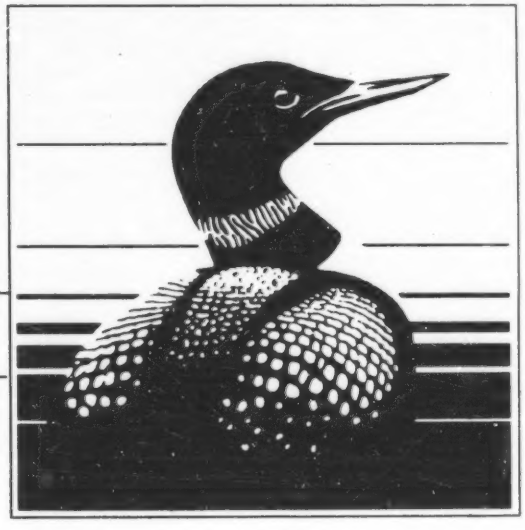
Multiresidue Methods for the Determination of Chlorinated Pesticides and Polychlorinated Biphenyls (PCBs) in Wildlife Tissues by Gas Chromatography/Mass Spectrometry

H.T. Won, M.J. Mulvihill and B.J. Wakeford

National Wildlife Research Centre 2001
Canadian Wildlife Service
Environmental Conservation Branch



Technical Report Series Number 335

Environment Canada | Environnement Canada
Canadian Wildlife Service | Service canadien de la faune

Canada

TECHNICAL REPORT SERIES
CANADIAN WILDLIFE SERVICE

This series of reports, established in 1986, contains technical and scientific information from projects of the Canadian Wildlife Service. The reports are intended to make available material that either is of interest to a limited audience or is too extensive to be accommodated in scientific journals or in existing CWS series.

Demand for these Technical Reports is usually confined to specialists in the fields concerned. Consequently, they are produced regionally and in small quantities; they can be obtained only from the address given on the back of the title page. However, they are numbered nationally. The recommended citation appears on the title page.

Technical Reports are available in CWS libraries and are listed in the catalogue of the National Library of Canada in scientific libraries across Canada. They are printed in the official language chosen by the author to meet the language preference of the likely audience, with a résumé in the second official language. **To determine whether there is significant demand for making the reports available in the second official language, CWS invites users to specify their official language preference. Requests for Technical Reports in the second official language should be sent to the address on the back of the title page.**

SÉRIE DE RAPPORTS TECHNIQUES
DU SERVICE CANADIEN DE LA FAUNE

Cette série de rapports donnant des informations scientifiques et techniques sur les projets du Service canadien de la faune (SCF) a démarré en 1986. L'objet de ces rapports est de promouvoir la diffusion d'études s'adressant à un public restreint ou trop volumineuses pour paraître dans une revue scientifique ou l'une des séries du SCF.

Ordinairement, seuls les spécialistes des sujets traités demandent ces rapports techniques. Ces documents ne sont donc produits qu'à l'échelon régional et en quantités limitées; ils ne peuvent être obtenus qu'à l'adresse figurant au dos de la page titre. Cependant, leur numérotage est effectué à l'échelle nationale. La citation recommandée apparaît à la page titre.

Ces rapports se trouvent dans les bibliothèques du SCF et figurent aussi dans la liste de la Bibliothèque nationale du Canada utilisée dans les principales bibliothèques scientifiques du Canada. Ils sont publiés dans la langue officielle choisie par l'auteur en fonction du public visé, avec un résumé dans la deuxième langue officielle. **En vue de déterminer si la demande est suffisamment importante pour produire ces rapports dans la deuxième langue officielle, le SCF invite les usagers à lui indiquer leur langue officielle préférée. Il faut envoyer les demandes de rapports techniques dans la deuxième langue officielle à l'adresse indiquée au verso de la page titre.**

101-02727/

MULTIRESIDUE METHODS FOR THE DETERMINATION OF CHLORINATED PESTICIDES AND POLYCHLORINATED BIPHENYLS (PCBS) IN WILDLIFE TISSUES BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

H.T. Won, M.J. Mulvihill and B.J. Wakeford

National Wildlife Research Centre
Canadian Wildlife Service
Environment Canada

Technical Report Series No. 335
Headquarters 2001
Canadian Wildlife Service

Issued under the Authority of the
Minister of Environment
Canadian Wildlife Service

© Minister of Public Works and Government Services Canada 2001
Catalogue No. CW69-5/335E
ISBN 0-662-28022-9

Copies may be obtained from:

Canadian Wildlife Service
National Wildlife Research Centre
100 Gamelin Blvd.
Hull, Québec, Canada
K1A 0H3

This report may be cited as:

Won, H.T., Mulvihill, M.J. and Wakeford, B.J. (2001) Multiresidue methods for the determination of chlorinated pesticides and polychlorinated biphenyls (PCBs) in wildlife tissues by Gas chromatography/Mass spectrometry. Technical Report Series No. 335E. Canadian Wildlife Service, Headquarters, Hull, Québec, Canada.

PREFACE

The Canadian Wildlife Service's National Wildlife Research Centre (NWRC) has been providing analytical determinations of chlorinated pesticides and polychlorinated biphenyl (PCB) congeners in wildlife tissues since 1986, in support to its National Wildlife Toxicology Program. Starting in 1997, all analytical data were determined using a quadrupole mass selective detector (MSD) coupled to the gas chromatograph instead of the electron capture detector (ECD) system that had been used up to that time. This change has allowed the unequivocal identification of a larger number of compounds.

This report provides a detailed description of the GC/MSD methodology along with information on analytical quality control. The first method, "***MET-CHEM-OC-04***", which describes the analysis of various biological matrices, is followed by "***MET-CHEM-OC-03***" which is applicable more specifically to adipose tissues and milk from the polar bear. The majority of the methodology used for the polar bear tissue is the same as that used for other tissues, the differences are mainly in the identification of particular compounds which are more commonly determined in the polar bear tissue.

Standard Operating Procedures (SOPs) specific to the organization are cited throughout this document. These SOPs are not readily available in the published literature but can be obtained from the authors, upon request.

The names of manufacturers, suppliers and trade names are included only to document the exact assay conditions adopted by the NWRC. Other equivalent products, instruments or reagents from other sources may also give satisfactory results.

PRÉFACE

Méthodes multi-résidus pour le dosage des pesticides organo-chlorés et des biphényles polychlorés (BPC) dans des tissus d'espèces sauvages.

Depuis 1986, le Centre national de la recherche faunique (CNRF) du Service canadien de la faune fournit des résultats d'analyse de pesticides organo-chlorés et de congénères de biphényles polychlorés (BPC), pour appuyer les projets de recherche reliés à son Programme national de surveillance des effets des produits toxiques sur les espèces sauvages. Depuis 1997, les résultats d'analyse sont obtenus utilisant un appareil de spectrométrie de masse de type quadripolaire couplé au chromatographe en phase gazeuse (GC/MSD), plutôt qu'un système de détection à capture d'électron (GC/ECD). Cette modification a permis l'identification unéquivoque d'un plus grand nombre de composés.

Le présent document donne une description détaillée de la méthode améliorée (GC/MSD) ainsi que l'information reliée aux activités de contrôle de qualité. La première méthode « ***MET-CHEM-OC-04*** », décrivant l'analyse de différentes matrices d'origine biologique, est suivie de la méthode « ***MET-CHEM-OC-03*** », applicable plus spécifiquement aux tissus adipeux et au lait d'ours polaire. Les étapes de la méthode pour l'analyse des tissus d'ours polaire sont très semblables à la méthode utilisée pour les autres tissus, les principales différences étant surtout reliées à l'identification des composés que l'on retrouve plus fréquemment dans les tissus d'ours polaires.

Tout au long du document on fait référence à des modes opératoires normalisés (« *SOPs* ») qui sont spécifiques à notre organisation. Ces procédures ne sont pas disponibles dans la littérature mais peuvent être obtenues en communiquant directement avec les auteurs.

Le nom des manufacturiers, fournisseurs et nom de commerce des produits sont inclus uniquement dans le but de documenter les conditions d'analyse précises utilisées par le CNRF. Des produits, instruments ou réactifs équivalents provenant d'autres sources peuvent aussi donner des résultats satisfaisants.

ABBREVIATIONS

DCM	dichloromethane
GC	gas chromatograph
GPC	gel permeation chromatography
HGQA	Herring gull eggs quality assurance material
MDC	minimum detectable concentration (3x noise level)
MSD	mass selective detector
MS	mass spectrometer
OC	organochlorine
PCB	polychlorinated biphenyl
PFTBA	perfluorotributylamine
SIM	selected ion monitoring
SRM	standard reference material
TCPM	tris(4-chlorophenyl) methanol

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the outstanding work of Ghislaine Sans Cartier in the preparation of this report. She has provided vital skills in editing data and text, collating information from the authors and has been instrumental in developing a clear layout of the material. We also wish to thank John Moisey and Mary Simon for the technical review of this document.

TABLE OF CONTENTS

PREFACE ..... *i*

PRÉFACE ..... *ii*

ABBREVIATIONS ..... *iii*

ACKNOWLEDGEMENTS ..... *iv*

MET-CHEM-OC-04 /

MULTIRESIDUE METHOD FOR THE DETERMINATION OF CHLORINATED PESTICIDES AND POLYCHLORINATED BIPHENYLS (PCBS) IN WILDLIFE TISSUES

1. SCOPE AND FIELD OF APPLICATION 1-1

2. REFERENCES 1-1

3. PRINCIPLES AND DEFINITIONS 1-2

4. REAGENTS, SOLUTIONS AND STANDARDS 1-3

4.1. REAGENTS
4.2. ADSORBENTS FOR SAMPLE CLEANUP
4.3. SOLUTIONS
4.4. STOCK STANDARDS
4.5. INTERMEDIATE STANDARD SOLUTIONS
4.6. WORKING STANDARD SOLUTIONS
4.7. MASS SPECTROMETER CALIBRATION STANDARD
4.8. OLIVE OIL STANDARD
4.9. QA REFERENCE MATERIAL
4.10. METHOD BLANK

5. AUXILIARY EQUIPMENT 1-11

5.1. GLASSWARE AND LABWARE
5.2. EQUIPMENT
5.3. INSTRUMENTATION

6. SPECIMEN OR SAMPLE HANDLING REQUIREMENTS 1-13

7. PROCEDURE 1-13

7.1. COLUMNS PREPARATION
7.2. EXTRACTION - EGGS AND TISSUE SAMPLES
7.3. EXTRACTION - PLASMA SAMPLES
7.4. SAMPLE CLEANUP BY GPC
7.5. SAMPLE CLEANUP BY FLORISIL COLUMN
7.6. GC OPERATING CONDITIONS
7.7. MSD OPERATING CONDITIONS
7.8. DATA ANALYSIS PARAMETERS
7.9. COMPOUND INFORMATION
7.10. MS TUNING

7.11. Analysis of Standards and Samples
7.12. Initial Calibration Curve
7.13. Moisture Determination
7.14. Lipid Determination - Egg and tissue samples
7.15. Lipid Determination - Plasma samples

8. EXPRESSION OF RESULTS 1-27

8.1. Calculation of Analyte Concentration
8.2. Calculation of Recovery for Labeled Internal Standards

9. REPRESENTATIVE DOCUMENTS 1-28

10. QUALITY CONTROL 1-34

10.1. MSD Tuning
10.2. Calibration Verification
10.3. Detection Limits and reporting limits
10.4. Ongoing Precision and Recovery
10.5. Accuracy
10.6. Method Blank
10.7. Target compound identification
10.8. Standard Operating Procedures
10.9. Data Validation
10.10. Method Validation
10.11. External Quality Control

11. CRITICAL CONTROL POINTS 1-38

TABLES

TABLE 1 - Supplier, catalogue number and concentrations of PCBs and organochlorines standards 1-8

TABLE 2 - Composition of OCs initial calibration standards (CS) in pg/µL 1-9

TABLE 3 - Composition of PCB congeners initial calibration standards (CS) in pg/µL 1-10

TABLE 4 - Chromatographic windows and characteristic ions for OCs analysis 1-19

TABLE 5 - Chromatographic windows and characteristic ions for PCBs analysis 1-19

TABLE 6 - Retention time and target ions for OC compounds, internal standard and normalization standard 1-20

TABLE 7 - Retention time and target ions for PCB compounds 1-21

FIGURES

FIGURE 1 - Flow diagram of extraction, cleanup and analysis of OCs and PCBs 1-26

FIGURE 2 - PFTBA spectra - AutoTune 1-28

FIGURE 3 - Initial calibration curve for PCB-118 1-29

FIGURE 4 - Chromatogram of OCs standards 1-30

FIGURE 5 - Chromatogram of PCBs standards (Aroclor mixture) 1-31

FIGURE 6 - Chromatogram of herring gull egg sample (analyzed for OCs) 1-32

FIGURE 7 - Chromatogram of herring gull egg sample (analyzed for PCBs) 1-33

MET-CHEM-OC-03 /

Multiresidue Method for the Determination of Chlorinated Pesticides and Polychlorinated Biphenyls (PCBs) In Polar Bear Tissues

1. SCOPE AND FIELD OF APPLICATION 2-1

2. REFERENCES 2-1

3. PRINCIPLES AND DEFINITIONS 2-2

4. REAGENTS, SOLUTIONS AND STANDARDS 2-2

4.1. Reagents
4.2. Adsorbents for Sample Cleanup
4.3. Solutions
4.4. Standards
4.5. QA Reference Material - Secondary Quantitation standard
4.6. Method Blank

5. AUXILIARY EQUIPMENT 2-4

5.1. Glassware and Labware
5.2. Equipment
5.3. Instrumentation

6. SPECIMEN OR SAMPLE HANDLING REQUIREMENTS 2-5

7. PROCEDURE 2-5

7.1. Columns Preparation
7.2. Extraction - Milk samples
7.3. Extraction - Adipose tissue samples (larger than 5 g - wet weight)
7.4. Extraction - Adipose tissue samples (biopsy samples)
7.5. Extraction - Low-lipid samples
7.6. Sample Cleanup by GPC
7.7. Sample Cleanup by Florisil Column
7.8. GC Operating Conditions
7.9. MSD Operating Conditions
7.10. Data Analysis Parameters
7.11. Compound Information
7.12. MS Tuning
7.13. Analysis of Standards and Samples

8. EXPRESSION OF RESULTS 2-14

8.1. Calculation of Analyte Concentration
8.2. Calculation of Recovery for Labeled Internal Standards

9. REPRESENTATIVE DOCUMENTS 2-15

10. QUALITY CONTROL 2-16

10.1. MSD Tuning
10.2. Calibration Verification

10.3. Detection Limits
10.4. Ongoing Precision and Recovery
10.5. Accuracy
10.6. Method Blank
10.7. Target compound identification
10.8. Standard Operating Procedures
10.9. Data Validation
10.10. External Quality Control

11. Critical Control Points **2-18**

TABLES

TABLE 1 - Chromatographic windows and characteristic ions **2-10**

TABLE 2 - Retention time and target ions for secondary quantitative standard (PBQ2) and internal standard **2-11**

TABLE 3 - Results table / Polar bear liver **2-15**

APPENDIX A /

Numbering of PCB congeners **A-1**

MULTIRESIDUE METHOD FOR THE DETERMINATION OF CHLORINATED PESTICIDES AND POLYCHLORINATED BIPHENYLS (PCBS) IN WILDLIFE TISSUES

1. SCOPE AND FIELD OF APPLICATION

The method that follows is applicable to the analysis of various matrices such as egg, liver, breast muscle and plasma for the determination of trace levels of chlorinated pesticides (OCs) and polychlorinated biphenyls (PCBs). There are specific contaminants which are usually targeted and they are listed in **Tables 6** and 7. Typical detection limits of 0.001 ppm (or less) are obtained for most compounds of interest. For the analysis of tissues and milk from polar bear (*Ursus maritimus*), a description of the methodology used is included in the second part of this report (method "MET-CHEM-OC-03").

2. REFERENCES

2.1. Wakeford, B. and Turle R. (1997) In-house reference materials as a means to quality assurance: The Canadian Wildlife Service Experience. In Reference Materials for Environmental Analysis. Clement, R.E., Keith, L.H. and Siu, M.K.W. (eds.), CRC Press, 205-231.

2.2. Zhu, J.P., Simon, M., Mulvihill, M.J. and Norstrom, R.J. (1994) Single fraction determination of PCBs and organochlorine contaminants by GC-MS using characterized tissue extracts as secondary standards. NWRC unpublished data.

2.3. Won, H. (1994) Evaluation of GC/MSD method for 21 organo chlorine standards for detection limit and linear range. NWRC internal report.

2.4. Won, H. (1994) Evaluation of GC/MSD method for some isomers in Aroclor (1242/1254/1260) (1:1:1) for detection limit and linear range. NWRC internal report.

2.5. Wakeford, B. (1998) Validation procedure for method MET-CHEM-OC-04A - Analysis of chicken egg homogenates fortified with organo chlorine substances and polychlorinated biphenyls. NWRC internal report CHEM-OC-97-34.

2.6. Norstrom, R.J. and Won, H.T. (1985) Long term preservation of egg and tissue homogenates for the determination of organochlorine compounds: Freezing versus freeze drying. *J. Assoc. Off. Anal. Chem.* 68(1), 129-135.

2.7. HP 5971A Mass selective detector hardware manual (HP 05971-90011) and MS ChemStation user's guide (HP G1034-90043).

2.8. O.I. Analytical (1996) Operating manual, Autoprep 1000™ chromatograph, Rev. 2.

2.9. Frings, C.S., Fendley, T. W., Dunn, R. T. and Queen, C. A. (1972) Improved determination of total serum lipids by the sulfo-phospho-vanillin reaction, *Clin. Chem.*, 18(7), 673-674.

2.10. NCP Quality Assurance Coordinator, May 12, 2000 report. National Water Research Institute, Burlington, Ontario.

3. PRINCIPLES AND DEFINITIONS

This method is intended for the analysis of lipophilic trace organic chemical contaminants in biological tissues. The first step, to extract the lipids from the tissue, is accomplished by extracting dried tissue homogenates with DCM/hexane or, in the case of plasma, by first denaturing it with formic acid prior to the DCM/hexane extraction. Chemicals of interest in the extracts are then separated from lipids and biogenic compounds by gel permeation chromatography (GPC), except for plasma extracts for which it is unnecessary. Any residual lipids are removed by Florisil column chromatography. Finally, the OCs and PCBs levels are determined via high resolution gas chromatography coupled to a mass spectrometry detection system. Identification of target analytes is accomplished by comparing GC retention times and specific mass fragments known to be present in the spectra of authentic compounds. Quantitation is accomplished by comparing the intensity of targeted mass fragments in specimen extracts to the same compounds in standard mixtures, injected separately on the GC/MSD system.

4. REAGENTS, SOLUTIONS AND STANDARDS

SAFETY PRECAUTIONS

⇒ The toxicity or carcinogenicity of each reagent and standard used in this method has not been precisely defined. Each chemical must be treated as a potential health hazard and exposure should be reduced to the lowest possible level.

⇒ Operations with toluene, DCM, hexane, methanol and acetone should be performed in a fume hood and dermal contact with solvents should be avoided.

⇒ Standards should always be opened and used in a fume hood. Handling of these compounds must be done only by qualified technical staff.

⇒ General safety rules and waste disposal procedures that apply to the Trace Organic Chemistry Laboratory must be followed (ref. Safety Manual).

⇒ Material Safety Data Sheets (MSDSs) for the products used in the assay must be read.

4.1. Reagents

4.1.1. Acetone, Omnisolv®, EM Science AX0116-1

4.1.2. Hexane, Omnisolv®, EM Science HX0296-1

4.1.3. Dichloromethane, Omnisolv®, EM Science DX0831-1

4.1.4. Methanol, Omnisolv®, EM Science MX0488-1

4.1.5. Toluene, Omnisolv®, EM Science TX0737-1

4.1.6. 2,2,4-Trimethylpentane (iso-octane), Omnisolv®, EM Science TX1389-1

4.1.7. Formic acid, AnalaR®, EM Science B10115

4.1.8. *o*-Phosphoric acid, 85%, HPLC grade, Fisher Scientific A260-500

4.1.9. Sulfuric acid, Fisher Scientific A510-500

4.1.10. Sodium sulfate, anhydrous granular (Na_2SO_4), EM Science ACS850-46

Wash 600 g of Na_2SO_4 in a glass column 3 cm ID x 50 cm long with 600 mL DCM/hexane (1:1), air dry in an open dish under the fume hood, heat 3 h at 400°C in a muffle furnace, cool and transfer in a tightly capped glass bottle. *Note:* If, after

heating, the sodium sulfate develops a grayish cast (due to the presence of carbon in the crystal matrix), discard that batch.

4.1.11. Ethyl alcohol, distilled in glass, EM Science EX0278-6

4.1.12. Vanillin, crystalline, Fisher Scientific V10-100

4.1.13. Olive oil, Acros Organic 41653-5000

4.1.14. De-ionized water from the Milli-RO / Milli-Q system (Millipore)

4.1.15. Helium, compressed bottled gas, Praxair, HE UHP SG 103168K

4.1.16. Nitrogen, compressed bottled gas, Praxair, N_2 PRE PURE SG 105411K

4.2. Adsorbents for Sample Cleanup

4.2.1. Envirobeads™ S-X3, Select (200-400 mesh), ATS Scientific 091-203

4.2.2. Florisil®, pesticide grade, 60-100 mesh, EM Science B28722-44

Transfer 750 to 800 g of Florisil into a large porcelain dish and heat to 650°C for 6 h in a muffle furnace. Reduce furnace to 130°C and heat overnight. Transfer a known amount of Florisil to a 4 L amber glass bottle. Cool with lid closed. Add 1.2% de-ionized water to the Florisil. Close bottle with Teflon lined lid and shake contents to break up lumps. Transfer to a roller and agitate for 24 h to equilibrate before use. ***Shelf life:*** 2 months. If a longer period has elapsed since preparation, dry Florisil overnight at 130°C, cool in closed container, add de-ionized water and equilibrate as described above.

4.3. Solutions

4.3.1. *DCM/hexane, 1:1 (v/v)*

4.3.2. *Vanillin reagent, 6 g/L* - Dissolve 6.0 g of vanillin in water in a 1 litre volumetric flask and dilute to volume. Transfer in amber bottle and store at room temperature. ***Shelf life:*** stable for two months.

4.3.3. *Phospho-vanillin reagent* - Add 350 mL of vanillin reagent (4.3.2) and 50 mL of water to a 2 litre beaker. Add 600 mL of concentrated *o*-phosphoric acid, with constant stirring. Transfer in amber bottle and store at room temperature. ***Shelf life:*** stable for two months.

4.4. Stock Standards

Note: Details are given in **Table 1** and list of PCB congeners (based on Ballschmiter's nomenclature) is given in **Appendix A**.

4.4.1. Isotopically-labeled $^{13}C_{12}$-PCBs prepared in n-Nonane - Cambridge Isotope Laboratories (CIL), certified solution: 40 ± 4 µg/mL

4.4.2. A mixture of isotopically-labeled $^{13}C_6$-chlorobenzene prepared in iso-octane - Cambridge Isotope Laboratories (CIL) certified solution: 100 ± 10 µg/mL

4.4.3. Native (unlabeled) organochlorines - solid compounds from various suppliers. Stock solutions prepared in iso-octane as described in 4.5.1

4.4.4. Aroclor 1242, Aroclor 1254 and Aroclor 1260 - US EPA and Monsanto. Stock solution (with the three Aroclors) prepared in iso-octane as described in 4.5.2

4.5. Intermediate Standard Solutions

Note: Refer to SOP-CHEM-PROC-05 for details concerning the preparation and storage of standard solutions.

4.5.1. *Organochlorines* - Accurately weigh about 10 mg (between 10 to 30 mg, depending on the compound) of each of the twenty-one organochlorines quantitative standards listed in **Table 1**. Dissolve with iso-octane in the same 100 mL volumetric flask and dilute to volume (final concentration for each compound varies from 100 to 300 µg/mL). Store in 125 mL Hypo-vial and cap with Mininert valve at 4°C.

Note: Hexachlorobenzene and nonachlor need to be dissolved in a small amount of toluene before dilution with iso-octane.

4.5.2. *Aroclors* - Accurately weigh about 1 g of each of the three Aroclor standards listed in **Table 1**. Dissolve with iso-octane in the same 100 mL volumetric flask and dilute to volume. Store in 125 mL Hypo-vial and cap with Mininert valve at 4°C.

4.6. Working Standard Solutions

4.6.1. *Internal standard spiking solution* - Add 500 µL of each of the 6 $^{13}C_{12}$-PCBs and 250 µL of the chlorobenzene cocktail (ref. **Table 1)** in a 10 mL volumetric flask and dilute to volume with toluene.

4.6.2. *Normalization standard* - Dilute 3 mL of the $^{13}C_{12}$-PCB-138 solution in 27 mL toluene. ***Note:*** Normalizing on area responses of the $^{13}C_{12}$-PCB-138 in samples and standards corrects for changes in the MSD sensitivity between injections and changes in the final sample volumes.

4.6.3. *Quantitation standards* -

4.6.3.1. Organochlorines - Pipette 3 mL of the solution containing 21 OCs (Section 4.5.1) in a 1 L volumetric flask and dilute to volume with iso-octane. Mix well and store at room temperature away from light and heat.

4.6.3.2. Aroclors (PCBs) - Pipette 1 mL of the solution of Aroclors (Section 4.5.2) to a 100 mL volumetric flask and dilute to volume with iso-octane. Mix well and store at room temperature away from light and heat. This solution contains 100 µg/mL of each Aroclors (i.e., 0.3 µg/µL total PCBs). Working standard is prepared by diluting 3 mL of this solution to 100 mL with iso-octane. The final solution concentration will be 9000 pg/µL total PCBs.

4.6.4. *Initial calibration standards* - Calibration standards (CS1 through CS5) for OCs are prepared by diluting the solution prepared in Section 4.5.1 in iso-octane, to produce the concentrations in the working range shown in **Table 2.** For the PCBs, calibration standards (CS1 through CS5) are prepared by diluting the solutions in Section 4.5.2 to obtain concentrations shown in **Table 3**, ranging from 900 pg/µL to 30 000 pg/µL total PCBs. ***Note:*** PCB congeners concentrations shown are based on actual quantitation of each congener in the Aroclor solutions used [2.2].

4.7. Mass Spectrometer Calibration Standard

Perfluorotributylamine (PFTBA), cat. no. 110234-0, PCR Inc., Gainesville, FL.

4.8. Olive Oil Standard

Note: Used for plasma lipid determination.

4.8.1. Add ca 150 mg of olive oil to a 10 mL volumetric flask and dilute to volume with ethyl alcohol. Mix well and store at 4°C. ***Shelf life:*** one month.

4.8.2. For each set of assays, prepare 4 calibration standards to have

concentration ranging from 30 to 150 mg/10 mL by diluting the olive oil stock solution in ethyl alcohol.

4.9. QA Reference Material

Herring gull (*Larus argentatus*) egg homogenate prepared in-house from eggs collected in 1989 from Lake Ontario and diluted with domestic chicken eggs, to adjust contaminant concentrations. ***Note:*** Details on the preparation of this quality assurance material is given in Wakeford 1997 [2.1].

4.10. Method Blank

Clean reference matrix carried out through the entire analytical procedure. ***Note:*** chicken egg is used when contaminant free material with the same matrix as the sample is not available.

TABLE 1 - Supplier, catalogue number and concentrations of PCBs and organochlorines standards

Internal Standards			Solution concentrations	
	Supplier	Cat. no.	Std. (µg/mL)	Working Std. (ng/µL)
$^{13}C_{12}$-PCB				
$^{13}C_{12}$-PCB-28	CIL	EC-1413	40	2
$^{13}C_{12}$-PCB-52	CIL	EC-1424	40	2
$^{13}C_{12}$-PCB-118	CIL	EC-1435	40	2
$^{13}C_{12}$-PCB-153	CIL	EC-1406	40	2
$^{13}C_{12}$-PCB-180	CIL	EC-1407	40	2
$^{13}C_{12}$-PCB-194	CIL	EC-1418	40	2
$^{13}C_6$-Chlorobenzene cocktail containing:	CIL	EM-1725-A		
$^{13}C_6$-1,2,4,5-tetrachlorobenzene			100	2.5
$^{13}C_6$-1,2,4,5-pentachlorobenzene			100	2.5
$^{13}C_6$-1,2,4,5-hexachlorobenzene			100	2.5
Normalization Standard				
$^{13}C_{12}$-PCB-138	CIL	EC-1436	40	4
Quantitation Standards				
Organochlorines			Std. (µg/mL)	Working Std. (pg/µL)
1,2,4,5-tetrachlorobenzene	Ultra Scientific	RCP-29	100	300
1,2,3,4-tetrachlorobenzene	Ultra Scientific	RCP-27	100	300
pentachlorobenzene	Ultra Scientific	RCP-30	100	300
hexachlorobenzene	US EPA	3920	100	300
alpha-hexachlorocyclohexane	US EPA	0620	100	300
beta-hexachlorocyclohexane	US EPA	0640	100	300
gamma-hexachlorocyclohexane	US EPA	0680	100	300
octachlorostyrene	private source	n/a	100	300
oxychlordane	US EPA	5200	300	900
trans-chlordane	US EPA	1240	200	600
cis-chlordane	US EPA	1220	200	600
trans-nonachlor	US EPA	5080	200	600
cis-nonachlor	private source	n/a	100	300
p,p'-DDE	US EPA	1860	300	900
p,p'-DDD	US EPA	1780	300	900
p,p'-DDT	US EPA	1920	300	900
photo-mirex	private source	n/a	100	300
mirex	US EPA	4720	200	600
heptachlor epoxide	US EPA	3880	100	300
dieldrin	US EPA	2380	200	600
tris(4-chlorophenyl)methanol	Lancaster	n/a	100	300
PCBs				
Aroclor-1242	US EPA	5703	10000	3000
Aroclor-1254	Monsanto	n/a	10000	3000
Aroclor-1260	Monsanto	n/a	10000	3000

TABLE 2 - Composition of OCs initial calibration standards (CS) in pg/µL

Organochlorines	CS1	CS2	CS3 *	CS4	CS5
1,2,4,5-tetrachlorobenzene	30	100	300	500	1000
1,2,3,4-tetrachlorobenzene	30	100	300	500	1000
pentachlorobenzene	30	100	300	500	1000
hexachlorobenzene	30	100	300	500	1000
alpha-hexachlorocyclohexane	30	100	300	500	1000
beta-hexachlorocyclohexane	30	100	300	500	1000
gamma-hexachlorocyclohexane	30	100	300	500	1000
octachlorostyrene	30	100	300	500	1000
oxychlordane	90	300	900	1500	3000
trans-chlordane	60	200	600	1000	2000
cis-chlordane	60	200	600	1000	2000
trans-nonachlor	60	200	600	1000	2000
cis-nonachlor	30	100	300	500	1000
p,p'-DDE	90	300	900	1500	3000
p,p'-DDD	90	300	900	1500	3000
p,p'-DDT	90	300	900	1500	3000
photo-mirex	30	100	300	500	1000
mirex	60	200	600	1000	2000
heptachlor epoxide	30	100	300	500	1000
dieldrin	60	200	600	1000	2000
tris(4-chlorophenyl)methanol	30	100	300	500	1000

* used daily to verify calibration and abundance ratios

TABLE 3 - Composition of PCB congeners initial calibration standards (CS) in pg/µL

PCBs		CS1	CS2	CS3 [a]	CS4	CS5
	PCB-18	24	81	244	406	812
	PCB-17	16	53	159	265	530
	PCB-16/32	16	52	156	259	519
	PCB-31	19	65	195	324	649
	PCB-28	21	70	209	348	695
	PCB-33/20	19	63	188	314	627
	PCB-22	9	29	87	145	290
	PCB-52	28	92	275	458	917
	PCB-49	12	40	120	200	400
	PCB-47/48	7	25	74	124	247
	PCB-44	17	58	173	289	577
	PCB-42	11	36	107	178	357
	PCB-64	13	44	132	221	442
	PCB-74	9	29	86	143	285
	PCB-70/76	22	73	218	363	725
	PCB-95	16	54	163	272	544
	PCB-66	24	82	245	408	816
	PCB-56/60	11	35	105	176	352
	PCB-92	6	19	57	95	189
	PCB-101/90	36	121	362	604	1208
	PCB-99	11	38	113	188	377
	PCB-97	9	31	94	157	314
	PCB-87	16	54	163	272	544
	PCB-85	5	17	51	85	170
	PCB-110	32	108	323	538	1076
	PCB-151	16	55	164	274	548
	PCB-149	37	124	371	619	1238
	PCB-118 [b]	23	78	235	391	782
	PCB-146	5	18	55	91	182
	PCB-153	40	133	400	666	1333
	PCB-105	12	41	122	204	408
	PCB-179	7	25	75	125	249
	PCB-141	11	36	107	178	356
	PCB-130	1	5	14	24	48
	PCB-176	2	8	25	41	82
	PCB-137	2	6	19	32	65
	PCB-158	5	16	48	80	160
	PCB-138	44	146	438	730	1460
	PCB-178	4	15	44	73	147
	PCB-187	18	59	177	296	591
	PCB-183	9	29	86	144	287
	PCB-128	6	19	56	93	187
	PCB-174	17	55	166	277	555
	PCB-177	9	30	90	150	299
	PCB-202	2	7	22	36	72
	PCB-171	4	13	38	64	128
	PCB-156	6	18	55	92	184
	PCB-200	2	6	19	31	62
	PCB-157	1	4	13	22	45
	PCB-172	3	8	25	42	83
	PCB-180	33	109	328	547	1093
	PCB-170/190	17	55	166	276	552
	PCB-201	8	26	78	130	260
	PCB-196/203	9	29	87	145	290
	PCB-208	2	6	17	29	57
	PCB-195	3	10	31	52	104
	PCB-207	2	5	16	27	54
	PCB-194	8	25	75	126	251
	PCB-206	2	7	22	36	73

[a] used daily to verify calibration and abundance ratios

[b] calibration curve shown in Figure 3

5. AUXILIARY EQUIPMENT

5.1. Glassware and Labware

5.1.1. Aluminum foil

5.1.2. Amber glass bottle - 1 L and 4 L with Teflon lined screwed cap

5.1.3. Autosampler vials - 2 mL, clear with red/orange crimp caps (HP 5181-3400)

5.1.4. C18 cartridge (Superclean ENVI-18, 6 mL tubes) Supelco 505706

5.1.5. Centrifuge tubes, graduated, glass - 15 mL with ℸ13 ground glass stopper

5.1.6. Centrifuge tubes, glass - 50 mL with screw-top

5.1.7. Column, glass, 1.0 cm ID x 24 cm long with Teflon™ stopcock and with ℸ19/22 outer joint at top of column (used for Florisil cleanup)

5.1.8. Column, glass - 2.1 cm ID x 35 cm long with Teflon™ stopcock and with ℸ24/40 outer joint at top of column (used for sample extraction)

5.1.9. Column glass - 3 cm ID x 50 cm long with Teflon™ stopcock and reservoir (for preparing the Na_2SO_4)

5.1.10. Cuvettes, acrylic, disposable (Sarstedt 67.738)

5.1.11. Flasks, flat bottom - 125, 250 and 500 mL all with ℸ24/40 outer joint

5.1.12. Flasks, pear shaped - 5 and 10 mL

5.1.13. Funnel, glass - 25 mm and 10 cm

5.1.14. Glass wool (Canadawide Scientific 54100-11), pre-washed with DCM/hexane (1:1) and air dried

5.1.15. GPC glass column - 3 cm ID x 60 cm long - Envirosep-ABC column assay (ABC Laboratories Inc., Columbia, MO, USA)

5.1.16. GPC tubes, 20 mL (ABC Laboratories Inc., Columbia, MO, USA)

5.1.17. Graduated cylinders, glass - 10, 50, 100 and 500 mL, and 1 L

5.1.18. Hand crimper, 8 mm - for crimping aluminum seals to autosampler vials (Chromatographic Specialties)

5.1.19. Mortars and pestles, glass

5.1.20. Pasteur pipets

5.1.21. Pipet, Eppendorf - 5-100 µL (with tips)

5.1.22. Pipets, glass, disposable - 0.5, 1, 2, 5 and 10 mL

5.1.23. Pipet, Rainin, with tips

5.1.24. Porcelain dish - 21.5 cm diameter

5.1.25. Reacti-vials - 1 and 5 mL and Hypo-vials - 125 mL with Mininert™ valve (Chromatographic Specialties Inc.)

5.1.26. Reservoir, glass - 125 mL, with ₹19/22 inner joint for glass column 5.1.7

5.1.27. Reservoir, glass - 250 mL, with ₹24/40 inner joint for glass column 5.1.8

5.1.28. Spatulas

5.1.29. Syringe, glass - 10 mL (B-D D3037)

5.1.30. Syringes, Hamilton™ - 10, 50, 100 and 250 µL

5.1.31. Volumetric flasks, glass - 10, 50 and 100 mL, and 1 L

5.1.32. Weighing aluminum dishes - disposable

5.2. Equipment

5.2.1. Analytical balance (Sartorius BP210D) and top-loading balance (Mettler PR700)

5.2.2. Vortex mixer

5.2.3. Rotary evaporator with water bath at ca 30°C (Büchi Rotavapor-R, Brinkman Instruments)

5.2.4. Refrigerated circulating bath at ca -4°C (Lauda RM20)

5.2.5. Drying oven (Fisher Scientific, Model 516 G)

5.2.6. Muffle furnace (Blue M Electric Company, Blue Island, Il, USA)

5.2.7. Centrifuge (Sorvall, SPX) with Sorvall Powerstat (Type 108-1164)

5.2.8. Visiprep solid phase extraction vacuum manifold (Supelco 57030) with Visidry drying attachment (Supelco 57100)

5.2.9. Water bath at 100°C (Blue M) - for plasma lipids

5.2.10. Water bath at 37°C (Precision Instruments) - for plasma lipids

5.2.11. Roller culture apparatus (Wheaton Instruments)

5.3. Instrumentation

5.3.1. GPC Autoprep™1000, with automatic sample loading and sample collection unit (from O.I. Analytical), with 23 sample ports (10 mL volume).

5.3.2. GC/MSD, Hewlett-Packard gas chromatograph (GC) 5890 Series II equipped with an autosampler (7673A), a Galileo Channeltron electron multiplier (5778) and linked to a Hewlett-Packard 5970 (or 5971A) mass selective detector (MSD) with MS ChemStation (HP G1034C, Rev. C.02.00); GC column: 30 m DB-5 (J&W) fused silica column, 0.25 mm ID, 0.25 μm film thickness (Chromatographic Specialties J1225032).

5.3.3. Spectrophotometer (Hewlett-Packard Diode Array, Model 8452A) - for plasma samples.

6. Specimen or Sample Handling Requirements

Samples provided to the Trace Organic Chemistry Laboratory are prepared as described in the Tissue Preparation Unit's standard operating procedure SOP-TP-PROC-07. These tissues were usually collected and preserved as recommended in the document "Protocol for Field Collection and Storage of Wild Birds for Biomarker Studies" (S. Trudeau, Biomarker Laboratory, NWRC, 1992).

7. Procedure

Flow chart that summarize procedures for sample extraction, cleanup and analysis is given in **Figure 1.**

7.1. Columns Preparation

7.1.1. *GPC Column*

Place 60 g Envirobeads ™ S-X3 in a 500 mL beaker. Cover the beads with DCM/hexane (1:1) and allow to swell overnight (a minimum of 12 h). Pack a GPC column (Section 5.1.15) with the pre-swelled beads. ***Note:*** This material generally makes 43 to 45 cm in column length.

7.1.2. *Florisil column*

Prepare fresh daily as described in Sections 7.5.1.-7.5.3.

7.2. Extraction - Eggs and tissue samples

Note: For plasma samples see Section 7.3.

7.2.1. Grind between 1.5 g to 3.0 g of the homogenized sample with ca 25 g of the treated anhydrous Na_2SO_4 (Section 4.1.10) in a glass mortar and pestle until a free-flowing mixture is obtained. ***Note:*** A sample size of 3 g is preferred. Include at least one standard reference material and a method blank sample with each batch of samples (a typical batch contains 10 samples).

7.2.2. Plug a 2.1 cm ID x 35 cm long glass column (Section 5.1.8) with some treated glass wool (Section 5.1.14), add about 1 cm Na_2SO_4. Pour ground sample mixture into the glass column and tap the column gently to settle the mixture. Add ca 0.5 cm Na_2SO_4. ***Note:*** Usually 5 columns are prepared at one time.

7.2.3. Place a 500 mL flat bottom evaporating flask under the column and open the stopcock. Rinse the mortar and pestle with DCM/hexane (1:1), and transfer the rinse onto the top of the column, repeat rinsing mortar and pestle three times. Close the stopcock.

7.2.4. Add enough DCM/hexane to cover the mixture and allow to soak for 30 min.

7.2.5. Attach the reservoir (Section 5.1.27) to the column and add 200 mL DCM/hexane (1:1). Elute at 5-10 mL/min (collecting the eluate in the 500 mL evaporating flask).

7.2.6. Evaporate the eluate to less than 5 mL on a rotary evaporator with water bath at ca 30°C, then quantitatively transfer into a 15 mL graduated centrifuge tube. Rinse the flask three times with ca 1 mL of DCM/hexane (1:1), adding the rinses to the centrifuge tube.

7.2.7. Add DCM/hexane (1:1) to have a sample concentration of 0.2 g/mL. For example with 3 g of tissue, adjust volume to 15 mL.

7.2.8. Transfer an aliquot equivalent to 0.5 g tissue into a pre-weighed aluminum dish, to be used for lipid determination (ref. Section 7.14).

7.2.9. Transfer an aliquot equivalent of 1 g of tissue into a GPC tube (Section 5.1.16). Spike the extract with 50 µL of the ^{13}C-labeled chlorobenzene/ PCB internal standard spiking solution (Section 4.6.1), and dilute to 10 mL with DCM/hexane (1:1). The sample is now ready for GPC cleanup.

7.3. Extraction - Plasma samples

7.3.1. Accurately weigh ca 3 g aliquot of the thawed plasma into a 50 mL screw-top centrifuge tube. Spike with 50 µL of the ^{13}C-labeled chlorobenzene /PCB internal standard spiking solution (Section 4.6.1). Mix the spiked plasma gently with a Vortex mixer, and let it stand for 30 min to equilibrate.

7.3.2. Add an equal volume of formic acid (e.g., 3 mL for 3 g plasma) to the spiked plasma in order to denature proteins; mix gently with Vortex mixer, and let it stand for 15 min.

7.3.3. Activate a C18 cartridge with two 6 mL portions of methanol followed by two 6 mL portions of de-ionized water, using the Visiprep solid phase vacuum manifold (Section 5.2.8).

7.3.4. Load the sample mixture (from 7.3.2) onto the activated C18 cartridge with suction at a flow rate of 6-7 mL/min. The polar interferences and lipids are not retained by the cartridge.

7.3.5. Dry the C18 cartridge thoroughly with a stream of nitrogen gas using a Visidry Drying attachment for ca 35 min. ***Note:*** Incomplete drying of the cartridge would result in sample loss.

7.3.6. Elute the analytes from the dried C18 cartridge with 3 x 2 mL of DCM/hexane (1:1). The sample is now ready for florisil cleanup (Section 7.6).

7.4. Sample Cleanup by GPC

Note: Calibrate GPC system when the column is changed, when channeling occurs, when column drying has occurred or when recoveries are not acceptable. Refer to the GPC operating manual [2.8] for specific instructions.

7.4.1. Load the GPC tubes (from 7.2.9) into the instrument.

7.4.2. Set GPC flow-rate at 5 mL/min of DCM/hexane (1:1), the "dump" time to 26 min, the "collect" time to 36 min and the wash time to 4 min. Initiate the operation of the GPC. ***Note:*** It is possible to load and run as many as 23 samples simultaneously (typically 12 are used). The sequence can be run overnight.

7.4.3. Evaporate the eluate from 7.4.2 to ca 3 mL on a rotary evaporator. The sample is now ready for Florisil column cleanup.

7.5. Sample Cleanup by Florisil Column

Florisil column cleanup is designed to isolate compounds of interest from any residual lipids.

7.5.1. Plug a 1 cm ID glass column (Section 5.1.7) with some treated glass wool (Section 5.1.14) and attach reservoir (Section 5.1.26).

7.5.2. With the stopcock in the close position, add 40 mL hexane to the reservoir and then 8 g of the de-activated Florisil (Section 4.2.2).

7.5.3. Open stopcock, and gently tap side of column to settle adsorbent and add ca 1 cm of Na_2SO_4. Close stopcock when solvent level is still slightly above the Na_2SO_4 layer. Discard rinse solvent.

7.5.4. Place 150 mL flat bottom evaporating flask at the end of column.

7.5.5. Load extract from 7.4.3 (or 7.3.6) on top of the Florisil column using Pasteur pipet. Rinse the flask 3-4 times with small portions of DCM/hexane (1:1). Add rinses to column, allowing solvent to drain to packing level in between rinses.

7.5.6. As the last rinse approaches top of Na_2SO_4 layer, add 95 mL of DCM/hexane (1:1) allow elution to proceed at ca 5 mL/min.

7.5.7. Concentrate eluate to less than 3 mL with rotary evaporator and quantitatively transfer into a 10 mL pear shaped flask. Rinse flask three times with 1 mL portions of iso-octane adding rinses to the flask.

7.5.8. Further concentrate the eluate to less than 400 µL with rotary evaporator. Quantitatively transfer the sample to an autosampler vial, previously marked at the 570 µL level. Rinse flask with a few drops of iso-octane, adding the rinses to the autosampler vial.

7.5.9. Add exactly 20 µL $^{13}C_{12}$-PCB-138 normalization standard (Section 4.6.2) to the autosampler vial using an Eppendorf pipet. Adjust the final volume to the 570 µL mark with iso-octane. ***Note:*** Exact volume is not critical, since normalization standard is used to quantify residue levels.

7.5.10. Cap vial and vortex until thoroughly mixed. The sample is ready to inject into the GC/MSD (see Section 7.12). ***Note:*** Each sample is injected twice. The first injection is designed to determine OC levels (see chromatographic windows and selected ions in **Table 4**). The second injection is designed to determine PCB levels (see chromatographic windows and selected ions in **Table 5**).

7.6. GC Operating Conditions

7.6.1. *Column*

- 30 m long DB-5 fused-silica column, 0.25 mm ID, 0.25 µm film thickness

7.6.2. *Injection information*

- Injection port temperature 250°C
- Source Auto
- Location Front
- Sample washes 0
- Sample pumps 5
- Sample volume 2 µL
- Viscosity delay 1 s
- Solvent washes 5A, 5B
- Splitless injection
- On column No
- Purge A on 1.5 min

7.6.3. *Oven temperature programme*

- For OCs: 100°C, hold 3 min; 20°C /min to 180°C; 5°C /min to 300°C
- For PCBs: 100°C, hold 3 min; 20°C /min to 180°C; 2.5°C /min to 300°C

7.6.4. *Carrier gas (He)*

- Head pressure 5-7.5 psi

7.7. MSD Operating Conditions

7.7.1. *MSD conditions*

- Electron impact (EI) ionization 70 eV (fixed)
- Acquisition mode selected ion monitoring mode (SIM)
- Dwell time (on each ion) 60 ms
- Source temperature 200°C
- Transfer line temperature 280°C

7.8. Data Analysis Parameters

- Calibration settings Reference window: 10.00%
- Non-reference window 5.00%

7.9. Compound Information

Typical retention time, target ion and qualifying ion are given in **Table 6 or 7** for analysis of OCs or PCBs respectively. The list is based on those compounds actually found in wildlife tissues.

TABLE 4 - Chromatographic windows and characteristic ions for OCs analysis

SIM Group	Start time (min)	Dwell time (ms)	Ions
Group 1	7.00	60	214, 216, 222, 224 [a]
Group 2	9.00	60	250, 252, 258 [a], 260
Group 3	11.00	60	183, 219, 284, 286, 292 [a], 294
Group 4	13.00	60	238, 268 [a], 270, 304 [a], 306, 345
Group 5	16.00	60	115, 237, 255, 263, 308, 339, 353, 380, 387
Group 6	17.60	60	237, 339, 373, 375
Group 7	18.40	60	246, 318, 373, 375, 407, 409
Group 8	19.00	60	237, 246, 318, 380
Group 9	20.10	60	165, 235, 338 [a], 340, 372 [a], 374, 407, 409
Group 10	21.70	60	165, 235, 237, 238, 372, 374
Group 11	23.50	60	237, 272, 406 [a], 408
Group 12	26.00	60	139, 251, 311, 346, 442 [a], 444

[a] ions selected to monitor the ^{13}C-labeled internal standards. Ion 372 is used to monitor the normalization standard ($^{13}C_{12}$-PCB-138)

TABLE 5 - Chromatographic windows and characteristic ions for PCBs analysis

SIM Group	Start time (min)	Dwell time (ms)	Ions
Group 1	15.00	60	256, 258
Group 2	18.00	60	256, 258, 268 [a], 290, 292, 304 [a]
Group 3	23.00	60	290, 292, 326, 328
Group 4	27.00	60	326, 328, 338 [a], 360, 362, 372 [a]
Group 5	31.00	60	360, 362, 394, 396, 372 [a]
Group 6	35.00	60	326, 328, 360, 362, 394, 396, 406 [a]
Group 7	40.00	60	428, 430, 442 [a], 464, 498

[a] ions selected to monitor the ^{13}C-labeled internal standards

TABLE 6 - Retention time and target ions for OC compounds, internal standard and normalization standard

Compound [a]	RT [b] (min)	RT window	Tgt [c] (m/z)	QI [d]	Abundance ratio [e]
Ocs (native)					
1,2,4,5-tetrachlorobenzene	7.73	7.23 - 8.23	216	214	77
1,2,3,4-tetrachlorobenzene	8.23	7.73 - 8.73	216	214	77
pentachlorobenzene	9.77	9.27 - 10.27	250	252	62
hexachlorobenzene	12.16	11.66 - 12.66	284	286	81
alpha-hexachlorocyclohexane	11.91	11.41 - 12.41	219	183	
beta-hexachlorocyclohexane	12.57	12.07 - 13.07	219	183	
gamma-hexachlorocyclohexane	12.78	12.28 - 13.28	219	183	
octachlorostyrene	17.10	16.60 - 17.6	308	380	
heptachlor epoxide	17.29	16.79 - 17.79	353	237	
oxychlordane	17.34	16.84 - 17.84	387	115	
trans-chlordane	18.08	17.58 - 18.58	375	373	88
cis-chlordane	18.59	18.09 - 19.09	375	373	88
trans-nonachlor	18.77	18.27 - 19.27	409	407	88
p,p'-DDE	19.23	18.73 - 19.73	246	318	
dieldrin	19.41	18.91 - 19.91	380	237	
p,p'-DDD	20.82	20.32 - 21.32	235	165	
cis-nonachlor	20.92	20.42 - 21.42	409	407	88
p,p'-DDT	22.01	21.51 - 22.51	235	165	
photomirex	23.65	23.15 - 24.15	238	272	
mirex	25.69	25.19 - 26.19	272	237	
tris(4-chlorophenyl)methanol	28.93	28.43 - 29.43	251	139	
^{13}C-internal standard					
$^{13}C_{12}$-1,2,4,5-tetrachlorobenzene	7.73	7.23 - 8.23	224	222	77
$^{13}C_{12}$-1,2,4,5-pentachlorobenzene	9.77	9.27 - 10.27	258	260	65
$^{13}C_{12}$-1,2,4,5-hexachlorobenzene	12.16	11.66 - 12.66	292	294	82
$^{13}C_{12}$-PCB-28	14.36	13.86 - 14.86	268	270	97
$^{13}C_{12}$-PCB-52	15.40	14.90 - 15.9	304	306	77
$^{13}C_{12}$-PCB-118	20.41	19.91 - 20.91	338	340	65
$^{13}C_{12}$-PCB-153	21.19	20.69 - 21.69	372	374	81
$^{13}C_{12}$-PCB-180	24.52	24.00 - 25.02	406	408	97
$^{13}C_{12}$-PCB-194	27.79	27.29 - 28.29	442	444	88
Normalization standard					
$^{13}C_{12}$-PCB-138	31.90	31.40 - 32.40	372		

[a] Abbreviations:

DDE - dichloro diphenyl dichloro ethylene

DDD - dichloro diphenyl dichloro ethane

DDT - dichloro diphenyl trichloro ethane

PCB - polychlorinated biphenyl

[b] Retention time. Oven program: 100°C, hold 3 min; 20°C/min to 180°C; 5°C/min to 300°C

[c] Target ion - Mass (m/z)

[d] Qualifying ion

[e] Abundance ratio - applicable only to ions within molecular ion clusters

TABLE 7 - Retention time and target ions for PCB compounds

Compound [a]	RT [b] (min)	RT window	Tgt [c] (m/z)	QI [d]	Abundance ratio [e]
PCBs (native)					
PCB-18	16.81	16.48 - 17.48	256	258	97
PCB-17	16.90	16.50 - 17.50	256	258	97
PCB-16/32	17.64	17.30 - 18.30	256	258	97
PCB-31	18.82	18.58 - 19.58	256	258	97
PCB-28	18.89	18.52 - 19.52	256	258	97
PCB-33/20	19.35	19.04 - 20.04	256	258	97
PCB-22	19.72	19.42 - 20.42	256	258	97
PCB-52	20.58	20.52 - 21.52	292	290	77
PCB-49	20.81	20.52 - 21.52	292	290	77
PCB-47/48	21.00	20.71 - 21.71	292	290	77
PCB-44	21.65	21.37 - 22.37	292	290	77
PCB-42	21.84	21.56 - 22.56	292	290	77
PCB-64	22.32	22.00 - 23.00	292	290	77
PCB-74	23.60	23.34 - 24.34	292	290	77
PCB-70/76	23.80	23.54 - 24.54	292	290	77
PCB-95	24.06	23.89 - 24.80	326	328	65
PCB-66	24.01	23.75 - 24.75	292	290	77
PCB-56/60	24.98	24.72 - 25.72	292	290	77
PCB-92	25.06	24.80 - 25.80	326	328	65
PCB-101/90	25.40	25.14 - 26.14	326	328	65
PCB-99	25.71	25.46 - 26.46	326	328	65
PCB-97	26.67	26.42 - 27.42	326	328	65
PCB-87	26.98	26.73 - 27.73	326	328	65
PCB-85	27.24	27.00 - 28.00	326	328	65
PCB-110	27.58	27.34 - 28.34	326	328	65
PCB-151	28.36	28.12 - 29.12	360	362	81
PCB-149	29.10	28.87 - 29.87	360	362	81
PCB-118	29.21	28.97 - 29.97	326	328	65
PCB-146	30.27	30.05 - 31.05	360	362	81
PCB-153	30.66	30.43 - 31.43	360	362	81
PCB-105	30.87	30.64 - 31.64	326	328	65
PCB-179	31.58	31.35 - 32.35	394	396	97
PCB-141	31.46	31.23 - 32.23	360	362	81
PCB-130	31.92	31.69 - 32.69	360	362	81
PCB-176	32.04	31.81 - 32.81	394	396	97
PCB-137	32.07	31.84 - 32.84	360	362	81
PCB-158	32.58	32.00 - 33.00	360	362	81
PCB-138	32.59	32.19 - 33.19	360	362	81
PCB-178	32.42	32.78 - 33.78	394	396	97
PCB-187	33.01	32.85 - 33.85	394	396	97
PCB-183	33.55	33.21 - 34.21	394	396	97
PCB-128	33.88	33.46 - 34.46	360	362	81
PCB-174	34.18	33.76 - 34.76	394	396	97
PCB-177	35.05	34.51 - 36.20	394	396	97
PCB-202	35.41	35.15 - 36.15	430	428	88
PCB-171	35.67	35.50 - 36.50	394	396	97
PCB-156	35.71	35.17 - 36.17	360	362	81
PCB-200	35.78	37.50 - 38.50	430	428	88
PCB-157	36.19	35.90 - 36.90	360	362	81

cont'd

TABLE 7 - continued

	Compound [a]	RT [b] (min)	RT window	Tgt [c] (m/z)	QI [d]	Abundance ratio [e]
PCBs (native) - cont'd						
	PCB-172	36.14	35.84 - 36.84	394	396	97
	PCB-180	36.49	36.70 - 37.70	394	396	97
	PCB-170/190	36.92	38.64 - 39.64	394	396	97
	PCB-201	38.86	38.52 - 39.52	430	428	88
	PCB-196/203	39.46	39.16 - 40.16	430	428	88
	PCB-208	39.83	39.5 - 40.5	464		
	PCB-195	41.74	41.58 - 42.58	430	428	88
	PCB-207	41.80	41.35 - 42.35	464		
	PCB-194	42.27	41.92 - 42.92	430	428	88
	PCB-206	43.14	42.81 - 43.81	464		
PCBs (^{13}C-internal standard)						
	$^{13}C_{12}$-PCB-28	18.88	18.58 - 19.58	268		
	$^{13}C_{12}$-PCB-52	20.56	20.29 - 21.29	304		
	$^{13}C_{12}$-PCB-118	29.19	28.97 - 29.97	338		
	$^{13}C_{12}$-PCB-153	30.64	30.43 - 31.43	372		
	$^{13}C_{12}$-PCB-180	36.90	36.70 - 37.70	406		
	$^{13}C_{12}$-PCB-194	43.13	42.94 - 43.94	442		
Normalization standard						
	$^{13}C_{12}$-PCB-138	31.90	31.40 - 32.40	372		

[a] Abbreviations:

PCB - polychlorinated biphenyl

[b] Retention time. Oven program: 100°C, hold 3 min; 20°C/min to 180°C; 2.5°C/min to 300°C

[c] Target ion - Mass (m/z)

[d] Qualifying ion

[e] Abundance ratio - applicable only to ions within molecular ion clusters

7.10. MS Tuning

Note: For detailed instructions on the operation of the instruments, consult the equipment operator's manuals [2.6].

7.10.1. Tune the mass spectrometer daily (prior to sample acquisition) with a PFTBA calibration standard (Section 4.7). Detailed procedure and acceptance criteria are described in SOP-CHEM-PROC-12.

7.10.2. Print hard copies of the tuning data. An example is given in **Figure 2**.

7.11. Analysis of Standards and Samples

7.11.1. In an autosampler vial, combine 500 µL of the OCs solution prepared in 4.6.3.1 with 50 µL of the ^{13}C-labeled chlorobenzenes/PCBs (4.6.1) and 20 µL of the $^{13}C_{12}$-PCB-138 (4.6.2). Cap the vial and vortex to ensure mixing. ***Note:*** Prepared just prior to injection - used for OCs quantitation.

7.11.2. In an autosampler vial, combine 500 µL of the Aroclor solution prepared in 4.6.3.2 with 50 µL of the ^{13}C-labeled chlorobenzenes /PCBs (4.6.1) and 20 µL of the $^{13}C_{12}$-PCB 138 (4.6.2). Cap the vial and vortex to ensure mixing. ***Note:*** Prepared just prior to injection - used for PCBs quantitation.

7.11.3. Establish the operating conditions given in Sections 7.7-7.9.

7.11.4. Prepare sequence injection table as described in SOP-CHEM-PROC-10. A typical sequence would consist of the following solutions:

- OCs quantitation standard (7.11.1)
- QA reference material
- Method blank
- Samples (a series of 10)
- OCs quantitation standard (7.11.1)
- PCBs quantitation standard (7.11.2)
- QA reference material
- Method blank
- Samples (a series of 10)
- PCBs quantitation standard (7.11.2)

7.11.5. Start the injection sequence.

7.11.6. Once the sequence is completed, calculate and print the results as described in SOP-CHEM-DOC-06.

7.12. Initial Calibration Curve

7.12.1. To calibrate the analytical system and determine linearity, inject sequentially, 2 µL of the calibration standard mixtures - CS1, CS2, CS3, CS4 and CS5 (Section 4.6.4), using the instrument conditions listed in 7.7-7.9. An example of calibration curve is given in **Figure 3.**

Note: Repeat this initial calibration whenever new calibration standard solutions are prepared or if the analytical acceptance criteria for the daily calibration verification standard (CS3) have not been met (Section 10.3).

7.13. Moisture Determination

7.13.1. Accurately weigh ca 0.5 g of sample (tissue homogenate) into a pre-weighed aluminum dish and record the weight to 5 decimal places.

7.13.2. Place the dish in a drying oven at 105°C for about two hours, until constant weight is obtained.

7.13.3. The calculation of the moisture content is as follows:

$$\% \text{ moisture} = 100 - (W_d/W_w) \times 100$$

where: W_d = weight of dry sample
W_w = weight of wet sample

7.14. Lipid Determination - Eggs and tissue samples

Note: Lipid levels are determined to allow calculations of contaminants based on lipid content instead of wet-weight. For plasma samples, see Section 7.15.

7.14.1. Allow solvent in the aluminum dish (prepared in Section 7.2.8) to evaporate to dryness in the fume hood.

7.14.2. Heat dish in oven at 105°C for 10 min.

7.14.3. Take dish out of the oven, allow to cool and reweigh. The difference in weight is the weight of lipids in the sample.

7.14.4. The calculation of the lipid content is as follows:

$$\% \text{ lipids} = (W_l \times V_l / W_{te} \times V_{tl}) \times 100$$

where:
W_l = weight of lipids (7.14.3)
Wte = weight of sample extracted
Vtl = total volume of extract
V_l = volume of extract used for lipid determination (7.2.8)

7.15. Lipid Determination - Plasma samples

7.15.1. Add 20 µL of water (blank), standard olive oil solution of different concentrations (Section 4.8.2) or plasma (unknown) to 15 mL glass centrifuge tubes. Add 0.20 mL of concentrated sulfuric acid to each tube, stopper and mix the contents well on a Vortex mixer.

7.15.2. Place all tubes in boiling water bath (Section 5.2.9) for 10 min (±1 min), then cool them in cold water for ca 5 min.

7.15.3. Add 10 mL of the phospho-vanillin reagent (Section 4.3.3) to each tube, stopper and mix well on a Vortex mixer. Incubate in a water bath adjusted to 37°C (±2°C) for 15 min.

7.15.4. Cool the tubes for ca 5 min and then, within 30 min, measure the absorbance at 540 nm using the following operating parameters:

- **Computer file path :** C:\HP8452
- **Mode :** F2 - Quantitation
- **Wavelength :** F1 - Single Wavelength (540,0)

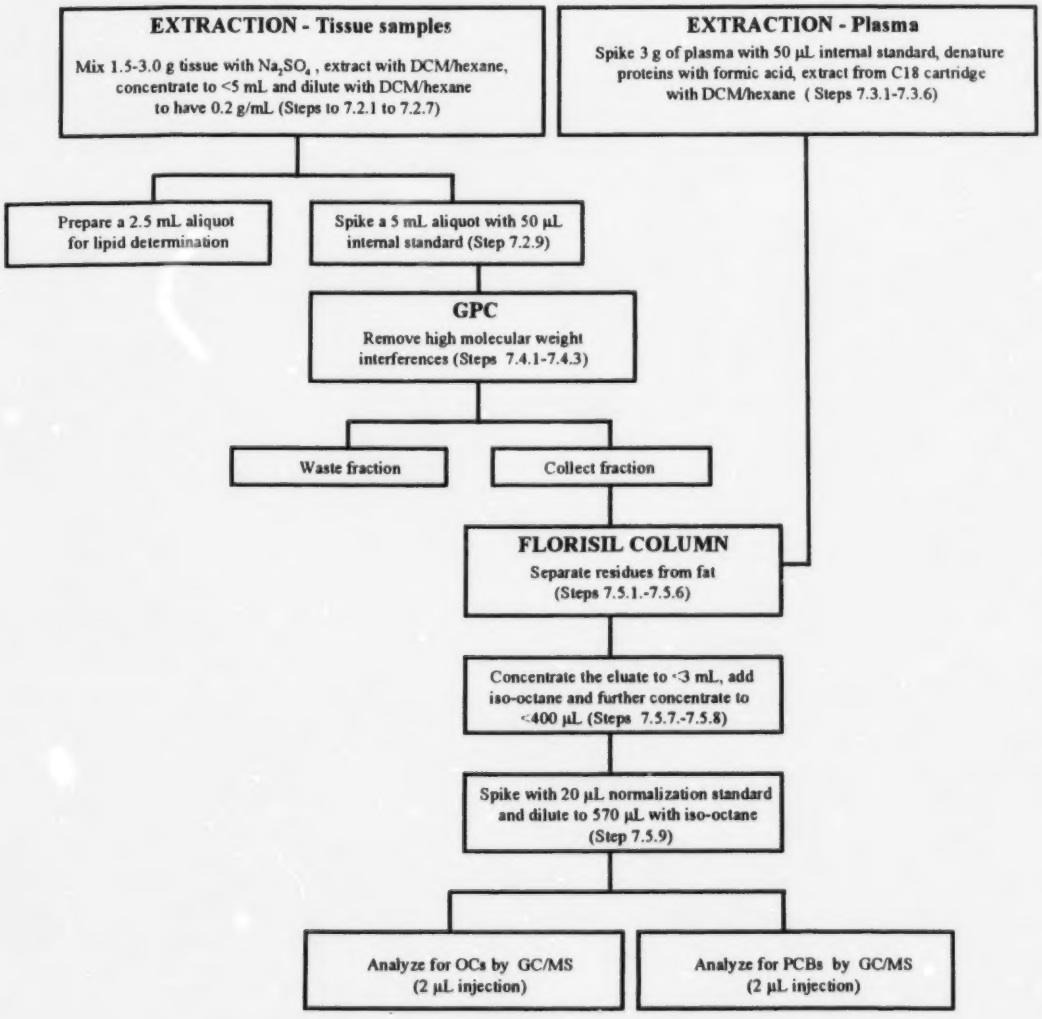
Note: Turn the spectrophotometer's lamp on one hour prior to the assay.

7.15.5. Press F8 to blank the instrument with the tube containing water.

7.15.6. Press F5 (Calibration) to access the "standard information table". Measure the absorbance of each of the standard olive oil solutions to create a calibration curve, specifying the concentration of each one. When readings are done, press F10 to exit.

7.15.7. Press F7 (Analysis) to access the "analysis results table". Measure the absorbance of the unknown plasma samples. The results of the absorbance and concentration will be automatically recorded based on the calibration curve. Print a hard-copy of the results by pressing F9.

FIGURE 1 - Flow diagram of extraction, cleanup and analysis of OCs and PCBs


EXTRACTION - Tissue samples
Mix 1.5-3.0 g tissue with Na_2SO_4, extract with DCM/hexane, concentrate to <5 mL and dilute with DCM/hexane to have 0.2 g/mL (Steps to 7.2.1 to 7.2.7)
EXTRACTION - Plasma
Spike 3 g of plasma with 50 µL internal standard, denature proteins with formic acid, extract from C18 cartridge with DCM/hexane (Steps 7.3.1-7.3.6)
Prepare a 2.5 mL aliquot for lipid determination
Spike a 5 mL aliquot with 50 µL internal standard (Step 7.2.9)
GPC
Remove high molecular weight interferences (Steps 7.4.1-7.4.3)
Waste fraction
Collect fraction
FLORISIL COLUMN
Separate residues from fat (Steps 7.5.1.-7.5.6)
Concentrate the eluate to <3 mL, add iso-octane and further concentrate to <400 µL (Steps 7.5.7.-7.5.8)
Spike with 20 µL normalization standard and dilute to 570 µL with iso-octane (Step 7.5.9)
Analyze for OCs by GC/MS (2 µL injection)
Analyze for PCBs by GC/MS (2 µL injection)

8. EXPRESSION OF RESULTS

8.1. Calculation of Analyte Concentration

$$C_S = [(A_S / A_{STD}) \times C_{STD}] \times [(V_I / V_F)] \times [(A_{NS\ in\ STD} / A_{NS\ in\ S})] \times [(V_{SF} / W_S)] \times 10^{-6}$$

where: C_S = analyte concentration in the sample in µg/g (wet weight)

A_S = area counts of analyte (PCB or OC) in the sample

A_{STD} = area counts of analyte in the injected standard (7.11.2 for PCBs or 7.11.1 for OCs)

C_{STD} = analyte concentration in the standard solution (4.6.3.2 for PCBs or 4.6.3.1 for OCs) in pg/µL

V_I = initial volume of the standard solution (4.6.3.2 for PCBs or 4.6.3.1 for OCs) used to prepare the quantitation standard mixture, in µL

V_F = final volume of the PCBs or OCs quantitation standard mixture, in µL

V_{SF} = final volume of the sample, in µL

$A_{NS\ in\ STD}$ = area counts of $^{13}C_{12}$-PCB-138 normalization standard in the PCBs or OCs quantitation standard mixture

$A_{NS\ in\ S}$ = area counts of $^{13}C_{12}$-PCB-138 normalization standard in the sample

W_S = sample weight in g (wet weight)

10^{-6} to convert in µg/g

Note: A Microsoft Excel spreadsheet is used to automatically calculate these values following sample acquisition on the GC/MSD.

8.2. Calculation of Recovery for Labeled Internal Standards

The percent recovery (%R) of labeled compounds is calculated as follows:

$$\%R = (A_{IS\ in\ S} / A_{IS\ in\ STD}) \times (A_{NS\ in\ S} / A_{NS\ in\ STD})$$

where: $A_{IS\ in\ S}$ = area counts of the labeled internal standard in the sample

$A_{IS\ in\ STD}$ = area counts of the labeled internal standard in the quantitation standard mixtures (7.11.1 for OCs and 7.11.2 for PCBs)

$A_{NS\ in\ STD}$ = area counts of $^{13}C_{12}$-PCB-138 normalization standard in the PCBs or OCs quantitation standard mixture

$A_{NS\ in\ S}$ = area counts of $^{13}C_{12}$-PCB-138 normalization standard in the sample

Note: Recoveries are automatically calculated by the instrument.

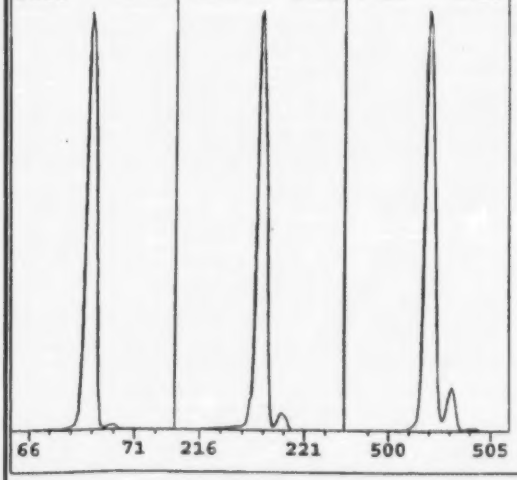
9. REPRESENTATIVE DOCUMENTS

FIGURE 2 - PFTBA spectrum - AutoTune

HP5971 Standard Spectra AutoTune

Instrument: msonly
Fri Jul 21 07:42:02 2000 C:\HPCHEM2\1\5971\ATUNE.U

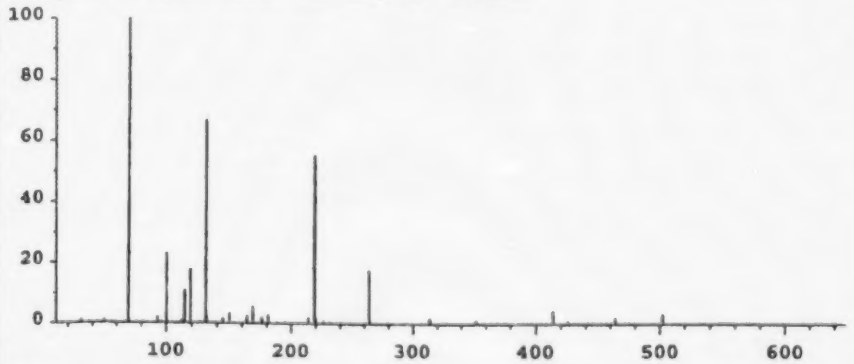
Mass	69.00	Mass	218.95	Mass	502.00
Ab	2609962	Ab	1572947	Ab	87280
Pw50	0.52	Pw50	0.52	Pw50	0.53



EMVolts	1835	AmuGain	483
Xray	8.6	AmuOffs	67
Emission	ON	Wid219	-0.030
MS Temp	184	TTI	OFF
Vacuum	51	DC Pol	NEG
Samples	16	Repeller	14.99
Averages	1	IonFocus	76.0
StepSize	0.10	EntLens	26.10
MassGain	-352	EntOffs	7.03
MassOffs	25	Filament	2

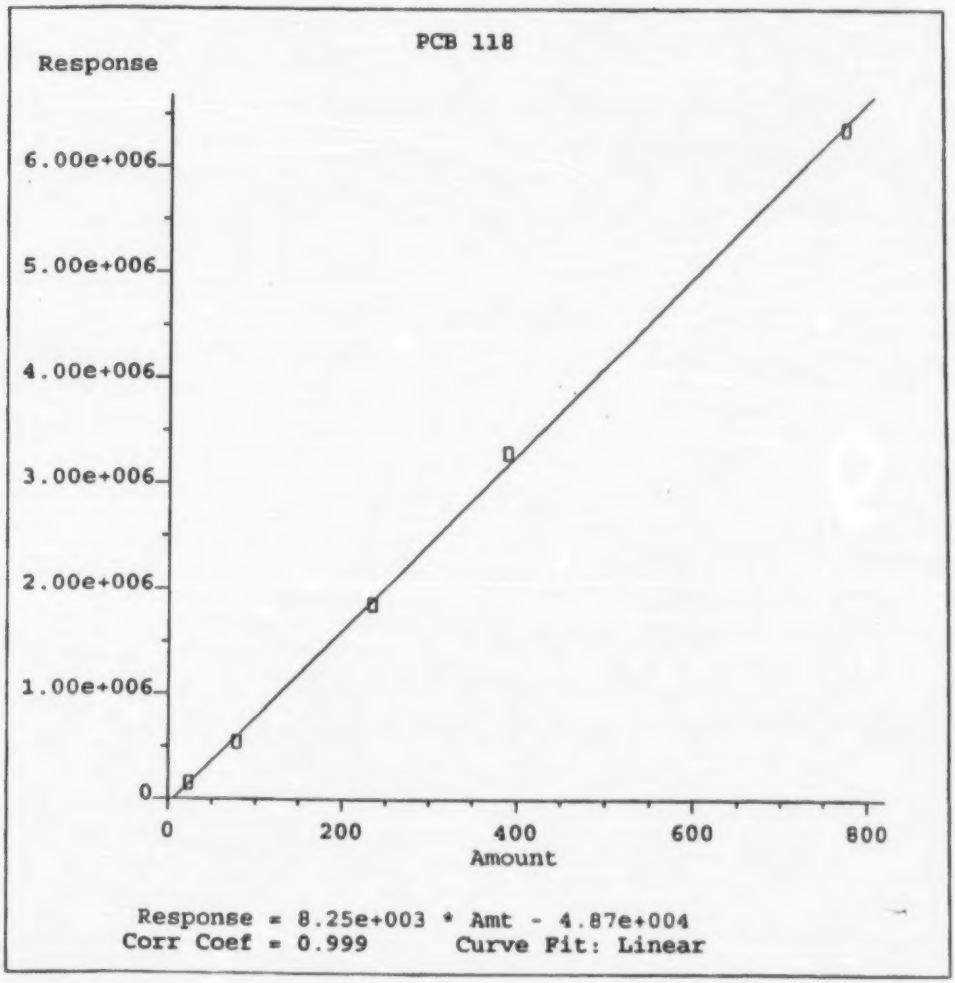
PFTBA OPEN

Scan: 10.00 - 650.00 Samples: 16 Thresh: 500 Step: 0.10
155 peaks Base: 69.05 Abundance: 2188288



Mass	Abund	Rel Abund	Iso Mass	Iso Abund	Iso Ratio
69.05	2188288	100.00	70.05	23040	1.05
218.90	1212416	55.40	219.90	49856	4.11
502.10	69096	3.16	503.00	6988	10.11

FIGURE 3 - Initial calibration curve for PCB-118



Method Name: C:\HPCHEM2\1\METHODS\5LEVPCB.M
Calibration Table Last Updated: Mon Jul 24 14:46:12 2000

Note: Actual concentrations shown in Table 3.

FIGURE 4 - Chromatogram of OCs standards

Name of file: C:\HPCHEM2\1\DATA\LINEAR\OC3A.D
Operator: H.W.
Sample: OC3A.D

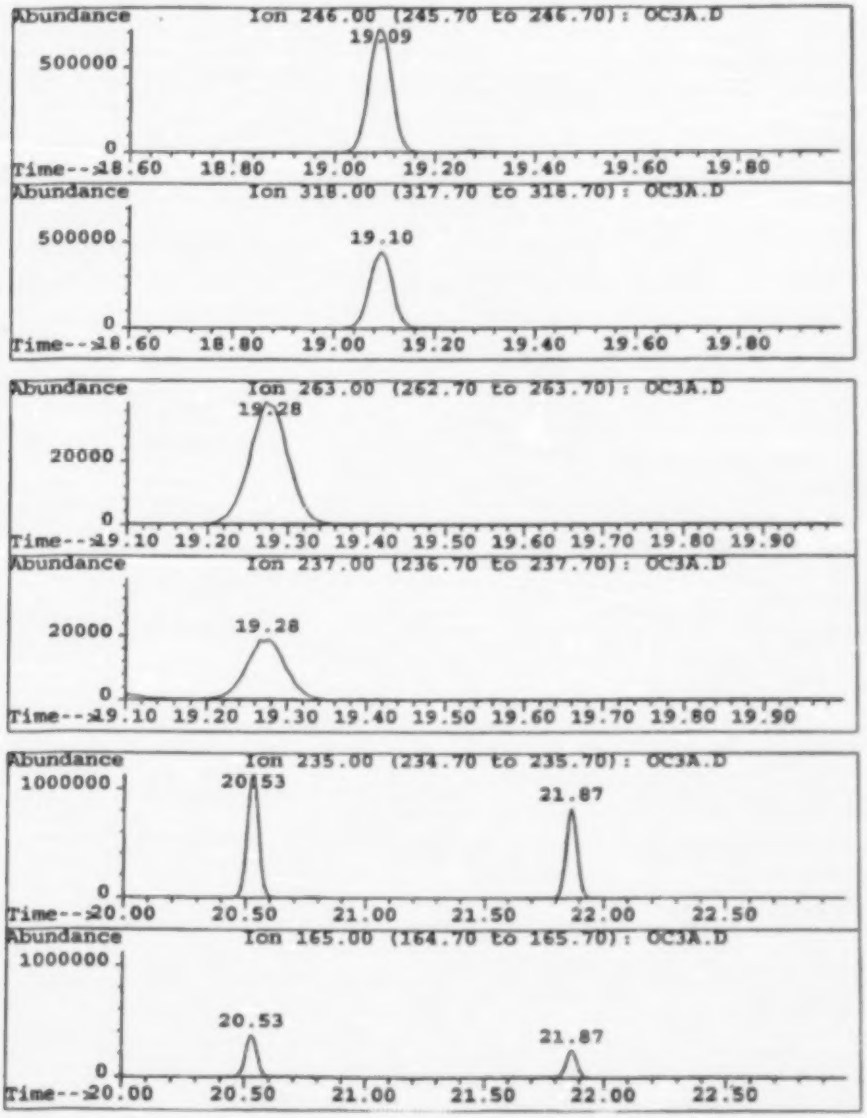


FIGURE 5 - Chromatogram of PCBs standards (Aroclor mixture)

Name of file: C:\HPCHEM2\1\DATA\LINEAR\PCB4A1.D
Operator: H.W.
Sample: PCB4A1.D

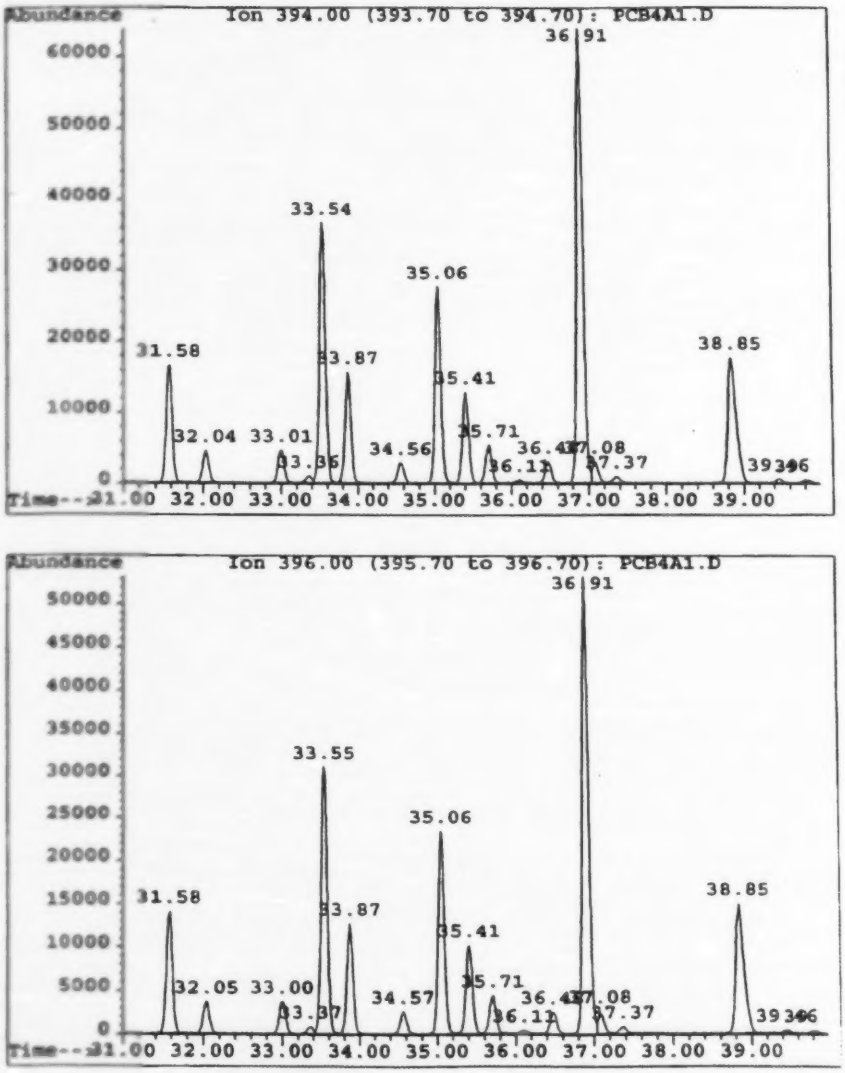


FIGURE 6 - Chromatogram of herring gull egg sample (analyzed for OCs)

Name of file: C:\HPCHEM2\1\DATA\0016A\E00765.D
Operator: H.W.
Sample: E00765.D

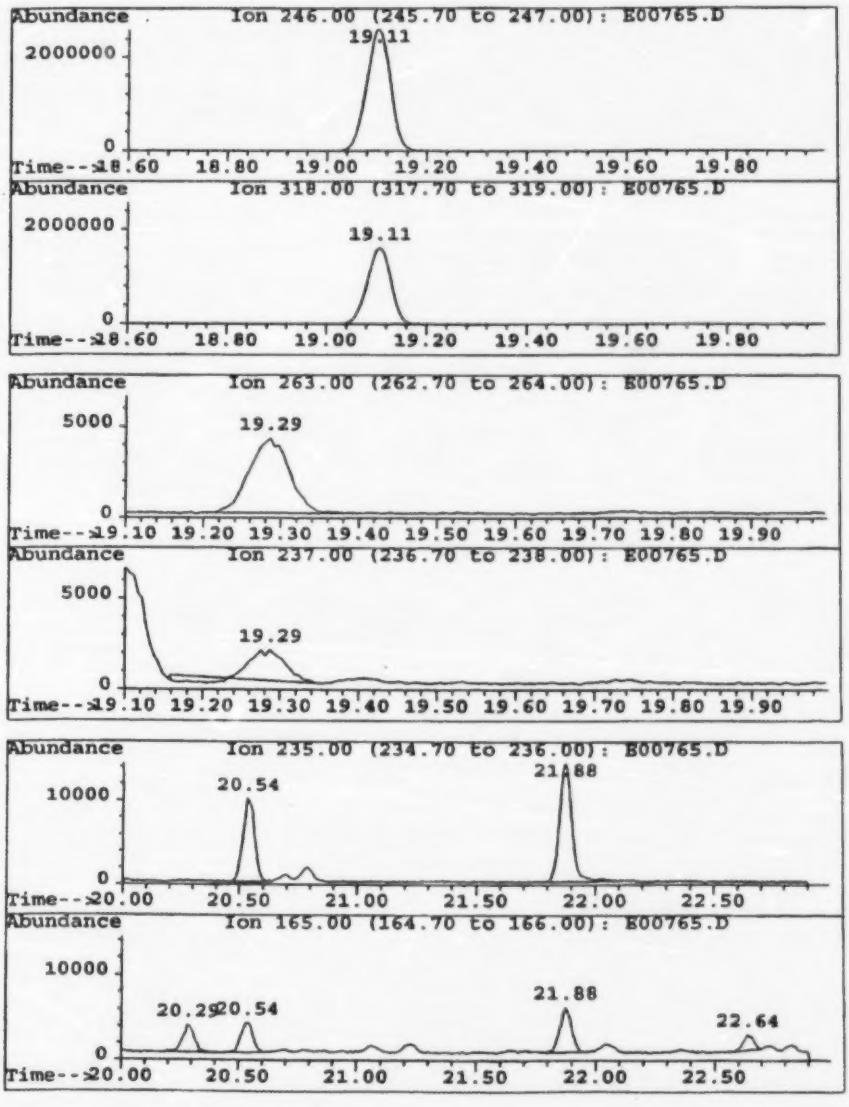
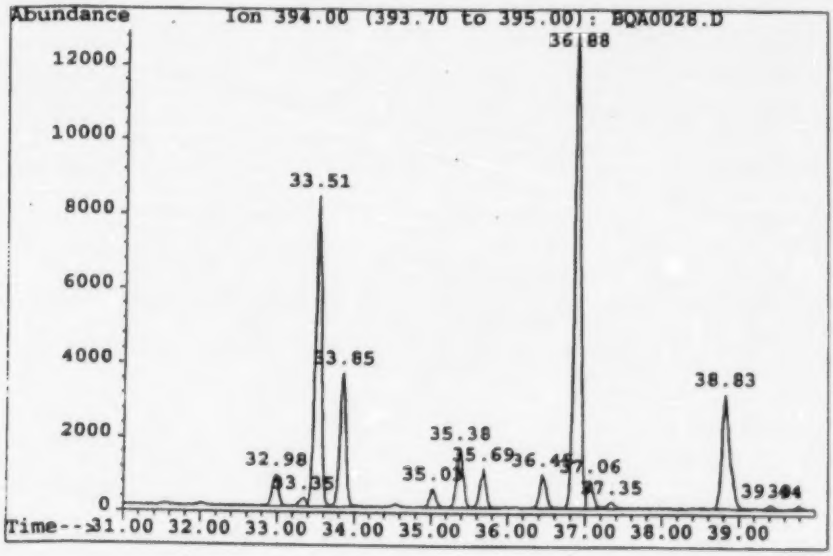
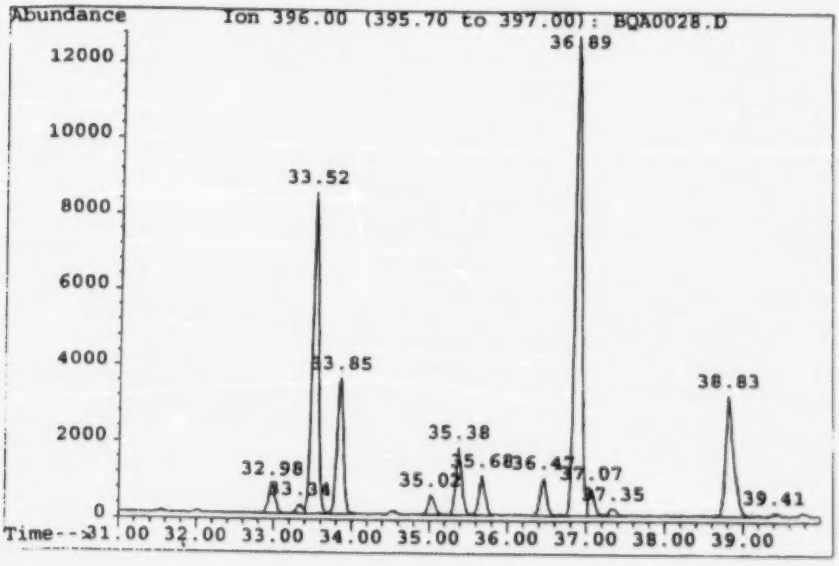


FIGURE 7 - Chromatogram of herring gull egg sample (analyzed for PCBs)

Name of file: C:\HPCHEM2\1\DATA\0028\BQA0028.D
Operator: H.W.
Sample: BQA0028.D





10. QUALITY CONTROL

10.1. MSD Tuning

The MSD is tuned weekly with the PFTBA calibration standard using the AutoTune program, and daily with the QuickTune program. The tuning of the instrument must meet the criteria for conformance outlined in SOP-CHEM-PROC-12 before sample analysis. Tune files are archived in a logbook.

10.2. Calibration Verification

A five-point initial calibration standard curve is made with the OCs and PCBs standard mixtures to cover the range of interest. This established calibration curve is verified daily, by analyzing a calibration verification standard (quantitation standard) having a mid-point concentration. The calculated concentration of each compound must be within 20% of its actual known value. The final concentration of any reportable compounds must be within the demonstrated linearity of the detector. If necessary, samples are diluted with iso-octane to meet the calibration range.

10.3. Detection Limits and Reporting Limits

A nominal or minimum detectable concentration (MDC) is usually described as the concentration of analyte which produces a signal in an instrument 3 times the average noise level. In this multi-residue method, it is possible but not practical to list the detection limits for each compound of interest. Variability between compounds arises due to varying background noise and response factors for each compound due to the different mass ions being monitored. The background noise is affected by several factors, such as tissue size and tissue type, and instrument effects such as column bleed and cleanliness of the source on the day of the analysis.

As a general rule, a detection limit of at least 0.001 PPM is achievable for all compounds. For the purposes of reporting data, no results less than this concentration are reported and a result of ND (not detected) appears in the Laboratory Services Section analytical test report. If a computed result falls in the range 0.0001 and 0.0009 PPM, the compound is defined as being detected but the result would be too variable to be reliable so a designation of TR (trace) is listed beside the compound in the final report.

10.4. Ongoing Precision and Recovery

An aliquot of the QA Reference Material (Herring gull eggs - Section 4.9) is analyzed along with each batch of samples. The concentration of the major compounds (PCB-52, PCB-66, PCB-101, PCB-110, PCB-149, PCB-118, PCB-146, PCB-153, PCB-138, PCB-187, PCB-180, PCB-170, PCB-201, PCB-203, HCB, *p,p'*-DDE, photo-mirex, mirex, oxychlordane, cis-nonachlor, heptachlor epoxide and dieldrin) is determined and the results are compared to the previously established acceptance limits (i.e., ± 2 SD of the long-term mean plotted in a Shewart chart - ref. SOP-CHEM-DOC-02).

To determine the degree of analyte loss during sample cleanup, each sample (including the PCBs and OCs standard mixtures 7.11.1 and 7.11.2) is spiked with ^{13}C-labeled chlorobenzenes/PCBs internal standard mixture (4.6.1). It is assumed that during sample cleanup, native compounds behave the same way as the labeled compounds. Analysis is accepted when the % internal standard recoveries for most PCBs and OCs are between 80% and 110%, and for the highly volatile compounds (eluting in the 1st, 2nd and 3rd chromatographic windows) are over 60%. The levels of contaminants reported in the analytical test report are not corrected for internal standard recoveries, but the recovery data are included.

10.5. Accuracy

To improve the accuracy of the method, the compound levels can be corrected based on the recovery of the ^{13}C-labeled chlorobenzenes/PCBs internal standard mixture (4.6.1). The majority of the chlorinated pesticides and PCB congeners levels can be corrected based on the average recovery of the five higher chlorinated ^{13}C-labeled PCB standards (i.e., PCB-52, PCB-118, PCB-153, PCB-180 and PCB-194). The more volatile compounds can be recovery-corrected using the recovery of their corresponding ^{13}C-labeled standards. For volatile compounds without ^{13}C-surrogates, retention time interpolation of recoveries with the ^{13}C-standards can be used.

The accuracy of the quantitation standards is verified annually with a second source standard (containing most of the congeners of interest) as described in SOP-CHEM-PROC-13.

10.6. Method Blank

A method blank is run with each batch of samples to determine the levels of contamination associated with the processing and analysis of samples. If

problems with blank exist, associated data are carefully evaluated and appropriate corrective actions are applied. Blank values are not subtracted from reportable values. A compound found in a blank and also in an associated sample is flagged in the analytical test report when present at a ratio of at least 5/1, sample to blank.

10.7. Target Compound Identification

Peak GC retention time must be within their pre-defined retention time windows.

All ions present in the standard mass spectrum at a relative intensity greater than 10% must be present in the sample spectrum.

The relative intensities of these ions must agree within ±20% between the standard and sample spectra (e.g., for an ion with an abundance of 50% in the standard spectrum, the corresponding sample ion abundance must be between 30% and 70%).

10.8. Standard Operating Procedures

Other related SOPs relevant to this analytical method:

- *SOP-CHEM-DOC-01*: for the use of log-books
- *SOP-CHEM-DOC-02*: for the creation of control charts for quality control samples
- *SOP-CHEM-DOC-04*: for the archival of gas chromatography data files
- *SOP-CHEM-DOC-05*: for the archival of analytical test reports
- *SOP-CHEM-PROC-01*: for pipets calibration
- *SOP-CHEM-PROC-03*: for balances calibration
- *SOP-CHEM-PROC-05* for the preparation and storage of standard solutions
- *SOP-CHEM-PROC-06*: for the monitoring of refrigerator's temperatures
- *SOP-CHEM-PROC-07*: for glassware cleaning
- *SOP-CHEM-PROC-10*: for running a sequence with the GC/MSD
- *SOP-CHEM-PROC-12*: for the tuning and calibration of the MSD
- *SOP-CHEM-PROC-13*: for verification of standard with a second source standard
- *SOP-CHEM-MAIN-04*: for the maintenance of the GC/MSD

10.9. Data Validation

Data validation is ensured by an internal quality assurance audit done by an independent reviewer (Head of the Laboratory Services Section), before the release of the analytical test report. Results of this verification are recorded on the "Data Validation Form for OC/PCBs Reports".

If large discrepancies in the analytical data between the specimens from close geographical areas are noted, then the raw data are examined - re-analysis of the sample aliquot may be indicated.

10.10.Method Validation

Some method validation data related to linearity and detection limits are compiled in internal reports [2.3 and 2.4]. Linear curves were obtained with OCs mixed standards solutions ranging from ca 10 to 1000 pg/µL. The limits of detection varied, depending on the compound, but ranged from a low of 10 pg/µL to 60 pg/µL for a 2 µL injection. For the PCBs, mixed Aroclor standards solutions ranging from 300 to 30 000 pg/µL (for the sum of the congeners), were analyzed. Most congeners responded linearly (R^2 between 0.991 and 1.00). The limits of detection varied depending on the congener but ranged from 1 pg for dichlorobiphenyls to 20 pg for nonachlorobiphenyl (PCB-206) for a 2 µL injection.

Recoveries experiments using spiked chicken eggs are detailed in Reference 2.5 - Internal report CHEM-OC-97-34.

10.11. External Quality Control

Whenever appropriate inter-laboratory check sample programs are available, the Laboratory participates in them. One example is "The Analysis of PCB Congeners and Organochlorines in Biota", which includes a variety of fish tissues provided as part of the Northern Contaminants Program (Indian and Northern Affairs Canada) [2.10].

11. CRITICAL CONTROL POINTS

A number of critical points are identified at various steps in Section 7. The following are repeated for emphasis:

Sample extracts must not be allowed to evaporate to dryness at anytime during the sample cleanup procedure. Several of the residue compounds, such as the chlorobenzenes and low chlorinated PCBs, are volatile. Reducing extracts to dryness will therefore result in loss of analytes. Evaporation of sample extracts using directed gas streams should be avoided for the same reasons, especially at volumes less than 1 mL. It is always preferable to use a rotary evaporator with a suitable flask volume, or sequence of flasks.

Sufficient mixing of the deactivated Florisil is necessary in order to assure equilibrium, before using the Florisil for sample cleanup.

To maintain the consistency and reproducibility of the GPC cleanup, always use the Envirobeads™ S-X3 described in 4.2.1.

Trace contaminant levels are determined by this method and the elimination of interferences is essential. They could occur through sample handling, reagents, solvents, instruments or labware.

MULTIRESIDUE METHOD FOR THE DETERMINATION OF CHLORINATED PESTICIDES AND POLYCHLORINATED BIPHENYLS (PCBS) IN POLAR BEAR TISSUES

1. SCOPE AND FIELD OF APPLICATION

The method that follows has been used in our laboratory for the analysis of various polar bear tissues such as fat, liver, muscle, lung, kidney, brain, gonads and milk for the chlorinated pesticides and polychlorinated biphenyls (PCBs) that are known to be present in the Canadian wildlife [2.4, 2.5]. Four different extraction methods are described to accommodate tissues with various lipids content, sample size and matrix. The specific contaminants targeted are listed in **Table 2**. Typical detection limits are in the range of 0.005 to 0.01 ppm.

2. REFERENCES

2.1. Zhu, J.P., Simon, M., Mulvihill, M.J. and Norstrom, R.J. (1994) Single fraction determination of PCBs and organochlorine contaminants by GC-MS using characterized tissue extracts as secondary standards. NWRC unpublished data.

2.2. Won, H. (1994) Evaluation of GC/MSD method for 21 organo chlorine standards for detection limit and linear range. NWRC internal report.

2.3. Won, H. (1994) Evaluation of GC/MSD method for some isomers in Aroclor (1242/1254/1260) (1:1:1) for detection limit and linear range. NWRC internal report.

2.4. Muir, D.C.G., Norstrom, R.J. and Simon, M. (1988) Organochlorine contaminants in Arctic marine food chains: Accumulation of specific PCB congeners and chlordane-related compounds. *Environ. Sci. Technol.*, 22, 1071-1079.

2.5. Norstrom, R.J., Simon, M., Muir, D.C.G. and Schweinsburg, R. (1988) Organochlorine contaminants in Arctic marine food chains: Identification, geographical distribution and temporal trends in polar bears. *Environ. Sci. Technol.* 22, 1063-1071.

2.6. Norstrom, R.J., Belikov, S.E., Born, E.W., Garner, G.W., Malone, B., Olpinski, S., Ramsay, M.A., Schliebe, S., Stirling, I., Stishov, M.S., Taylor, M.K., Wiig, Ø. (1998) Chlorinated hydrocarbon contaminants in polar bears from eastern Russia, North America, Greenland, and Svalbard: Biomonitoring of Arctic pollution. *Arch. Environ. Contam. Toxicol.* 35, 354-367.

2.7. HP 5970B Mass selective detector hardware manual (05970-90049) and MS ChemStation user's guide (HP-G1034-90043).

2.8. O.I. Analytical (1992) Operating manual, Autoprep 1000™ chromatograph, Rev. 2.

2.9. Zhu, J.P. (1993) Comparability of analytical data for PCBs and chlorinated pesticides: Review of polar bear fat extract results. NWRC internal report.

3. PRINCIPLES AND DEFINITIONS

Tissues are dried out with sodium sulfate and the residues are extracted with DCM/hexane. Milk samples are first denatured with methanol, and then extracted with DCM/hexane. Removal of lipids and biogenic compounds from the lipid extracts is done by gel permeation chromatography (GPC). The eluant is further cleaned up by Florisil column chromatography. Finally, the OCs and PCBs are determined via high resolution gas chromatography coupled to a mass spectrometry detection system. Identification of target analytes is accomplished by comparing GC retention times and specific mass fragments known to be present in the spectra of authentic compounds. Quantitation is accomplished by comparing the intensity of targeted mass fragments in specimen extracts to the same compounds in standard mixtures or in polar bear reference material (Section 4.5) previously characterized at NWRC [2.1].

4. REAGENTS, SOLUTIONS AND STANDARDS

SAFETY PRECAUTIONS

⇒ The toxicity or carcinogenicity of each reagent and standard used in this method has not been precisely defined. Each chemical must be treated as a potential health hazard and exposure should be reduced to the lowest possible level.

⇒ Operations with toluene, DCM, hexane, methanol and acetone should be performed in a fume hood and dermal contact with solvents should be avoided.

⇒ Standards should always be opened and used in a fume hood. Handling of these compounds must be done only by qualified technical staff.

⇒ General safety rules and waste disposal procedures that apply to the Trace Organic Chemistry Laboratory must be followed (ref. Safety Manual).

⇒ Material Safety Data Sheets (MSDSs) for the products used in the assay must be read.

4.1. Reagents

4.1.1. Acetone, Omnisolv®, EM SCIENCE CAAX0116-1

4.1.2. Hexane, Omnisolv®, EM SCIENCE CAHX0296-1

4.1.3. Dichloromethane, Omnisolv®, EM SCIENCE CADX0831-1

4.1.4. Methanol, Omnisolv®, EM SCIENCE CAMX0488-1

4.1.5. Toluene, Omnisolv®, EM SCIENCE CATX0737-1

4.1.6. Diethyl ether, anhydrous, Omnisolv®, EM SCIENCE CAACS291-76

4.1.7. 2,2,4-Trimethylpentane (iso-octane), Omnisolv®, EM SCIENCE CATX1389-1

4.1.8. Sodium sulfate, anhydrous granular, (Na_2SO_4), EM SCIENCE ACS850-46

Wash 600 g of Na_2SO_4 in a glass column 3 cm ID x 50 cm long with 600 mL DCM/hexane (1:1), air dry in an open dish under the fume hood, heat 3 h at 400°C in a muffle furnace, cool and transfer in a tightly capped glass bottle. ***Note:*** If, after heating, the sodium sulfate develops a greyish cast (due to the presence of carbon in the crystal matrix), discard that batch.

4.1.9. De-ionized water from the Milli-RO / Milli-Q system (Millipore)

4.1.10. Helium, compressed bottled gas, Praxair, HE UHP SG 103168K

4.1.11. Nitrogen, compressed bottled gas, Praxair, N_2 PRE PURE SG 105411K

4.2. Adsorbents for Sample Cleanup

See Section 4.2 of MET-CHEM-OC-04.

4.3. Solutions

4.3.1. *DCM/hexane, 1:1 (v/v)*

4.3.2. *Potassium oxalate 0.6 M* - Dissolve 55.2 g in de-ionized water and dilute to 500 mL.

4.4. Standards

Internal standard spiking solution and normalization standard as described in Sections 4.6.1 and 4.6.2 of MET-CHEM-OC-04.

4.5. QA Reference Material - Secondary Quantitation Standard

Since certified reference material with the same matrix does not exist, adipose tissue from polar bear prepared in-house is used. The current reference material used, identified as PBQ2, is from polar bear PB-142 collected in 1984 in the Canadian Arctic. Details about preparation and quantitation of the extracts is described in Norstrom et al. [2.6].

4.6. Method Blank

See Section 4.10 of MET-CHEM-OC-04.

5. AUXILIARY EQUIPMENT

5.1. Glassware and Labware

As described in Sections 5.1 of MET-CHEM-OC-04 with the following exceptions:

a) Item 5.1.4 (C18 cartridges) are not used

b) Filter disks - 0.2 µm (13 mm diam.), attached to a 5 mL syringe, are needed for adipose tissue biopsy samples (ref. Section 7.4.8).

5.2. Equipment

As described in Sections 5.2 of MET-CHEM-OC-04 with the following exceptions:

a) Items 5.2.8-5.2.10 (Visiprep solid phase extraction vacuum and water baths at 100°C and 30°C) are not used.

b) Omni-mixer homogenizer with 400-mL cup - Diamed (Sorvall 17105) is used for large adipose tissue samples (Section 7.3.1).

5.3. Instrumentation

See Sections 5.3.1-5.3.2 of MET-CHEM-OC-04.

6. Specimen or Sample Handling Requirements

Samples are prepared as described in the Tissue Preparation Unit's standard operating procedure SOP-TP-PROC-07. Collection and preservation protocols are described in Norstrom et al [2.6] and in "Protocol for Field Collection and Storage of Wild Birds for Biomarker Studies" (S. Trudeau, Biomarker Laboratory, NWRC, 1992).

7. Procedure

7.1. Columns Preparation

See Sections 7.1.1 and 7.5.1-7.5.3 of MET-CHEM-OC-04.

7.2. Extraction - Milk samples

7.2.1. Accurately weigh 3.00 g of milk in a 50 mL screw-top centrifuge tube and spike with 50 µL of the ^{13}C-labeled chlorobenzene/PCB internal standard spiking solution.

7.2.2. Add 3 mL of the $K_2C_2O_4$ solution (Section 4.3.2) to the spiked milk sample and mix with Vortex mixer.

7.2.3. Add 3 mL of methanol to the mixture, and let it stand for 15 min. Mix thoroughly with Vortex and let it stand for another 30 min.

7.2.4. Add 6 mL diethyl ether and 6 mL hexane. Vortex the mixture.

7.2.5. Centrifuge for 2 min at ca 2000 RPM to separate the organic and the aqueous phases.

7.2.6. Transfer the organic layer (top layer) with a Pasteur pipet into a 125 mL round bottom flask. Repeat the extraction procedure (steps 7.2.4-7.2.5) two more times.

7.2.7. Add 15 mL hexane and ca 1 g of anhydrous Na_2SO_4 (Section 4.1.8) to the combined extracts in order to absorb any residual moisture. Let

stand for 15 min.

7.2.8. Concentrate the extract to less than 5 mL using the rotary evaporator with the water bath adjusted to ca 30°C.

7.2.9. Quantitatively transfer the extract into a graduated 15 mL centrifuge tube and dilute to 10 mL with DCM/hexane solution (1:1).

7.2.10. Transfer a 1 mL aliquot (equivalent to 0.3 g sample) in a pre-weighed aluminum dish, to be used for lipid determination as described in Section 7.14 of MET-CHEM-OC-04.

7.2.11. The rest of the extract is ready for GPC cleanup. ***Note***: since 10% of the sample is removed for lipid determination, the internal standard % recovery measured will have to be corrected (see Section 10.4).

7.3. Extraction - Adipose tissue samples (larger than 5 g - wet weight)

7.3.1. Accurately weigh ca 5 g of sample (still frozen) in a 400 mL Sorval cup. Add 50 mL DCM/hexane (1:1) and homogenize with Omni mixer for ca 10 min.

7.3.2. Plug the end of a powder funnel with some treated glass wool (Section 5.1.12) and add ca 25 g of Na_2SO_4.

7.3.3. Place a 250 mL round bottom evaporating flask under the funnel.

7.3.4. Decant the homogenized sample mixture onto the Na_2SO_4, keeping the connective tissue in the Sorval cup.

7.3.5. Add another 50 mL DCM/hexane (1:1) in the cup and homogenize for ca 10 min. Decant the solvents onto the Na_2SO_4.

7.3.6. Repeat step 7.3.5 and transfer solvents and connective tissue on top of Na_2SO_4 layer.

7.3.7. Rinse funnel with 20 mL DCM/hexane (1:1).

7.3.8. Evaporate the eluate to less than 5 mL on a rotary evaporator with water bath at ca 30°C, then quantitatively transfer into a 12 mL graduated centrifuge tube. Rinse the flask three times with ca 1 mL of DCM/hexane (1:1), adding the rinses to the centrifuge tube.

7.3.9. Adjust volume to 10 mL with DCM/hexane (1:1) to have a sample concentration of 0.5 g/mL.

7.3.10. Transfer a 1 mL aliquot (equivalent to 0.5 g tissue) in a pre-weighed aluminum dish, to be used for lipid determination (ref. Section 7.14 of MET-CHEM-OC-04).

7.3.11. Pipette a 2 mL aliquot into a GPC tube. Spike the extract (equivalent to 1 g tissue - wet weight) with 50 µL of the ^{13}C-labeled chlorobenzene/ PCB internal standard spiking solution, and dilute to 10 mL with DCM/hexane (1:1). The sample is now ready for GPC cleanup.

7.3.12. Transfer the remaining extract from 7.3.9 in a 10 mL sealed ampule for future use.

7.4. Extraction - Adipose tissue samples (biopsy samples)

7.4.1. Accurately weigh ca 100 mg (or less) of fat, previously detached from the surface skin and hair, into a pre-weighed 20 mL scintillation vial. *Note:* Separate skin from the fat with a sharp scalpel while the biopsy sample is still partially frozen to facilitate the task.

7.4.2. Add ca 1 g of the treated anhydrous Na_2SO_4 and re-weigh the vial.

7.4.3. Grind the mixture with a stainless steel spatula. Cover vial with aluminum foil (leaving spatula in the vial) and allow to stand for 30 min.

7.4.4. Spike the mixture with 20 µL of the ^{13}C-labeled chlorobenzene/PCB internal standard spiking solution.

7.4.5. Add 5 mL of DCM/hexane (1:1) and allow to stand for 30 min. Transfer extracting solvents with a Pasteur pipet in into a 125 mL round bottom flask.

7.4.6. Repeat extraction (Step 7.4.5) three more times, combining the extracts in the 125 mL flask. *Note:* Thoroughly mix after each addition of fresh solvents to ensure complete lipid extraction.

7.4.7. Concentrate the extract to less than 2 mL using the rotary evaporator with the water bath adjusted to ca 30°C.

7.4.8. Filter the concentrated extract through a 0.2 µm filter disk attached to a

5 mL syringe. Rinse the filter 3 times with 1 mL DCM/hexane (1:1), combining all fractions in a GPC tube. The sample is ready for GPC cleanup.

7.5. Extraction - Low-lipid samples

Note: This extraction method is used for low-lipid samples such as liver, muscle, kidney, gonad, brain and lung.

See Sections 7.2 of MET-CHEM-OC-04

7.6. Sample Cleanup by GPC

Spiked sample extract (from 7.2.11, 7.3.11, 7.4.8 or 7.5) is injected into one loop of the GPC as described in Section 7.4 of MET-CHEM-OC-04.

7.7. Sample Cleanup by Florisil Column

As described in Sections 7.5.1-7.5.4 of MET-CHEM-OC-04 and then:

7.7.1. Load extract obtained after GPC cleanup step on top of the Florisil column using Pasteur pipet. Rinse the flask 3-4 times with small portions of DCM/hexane (1:1). Add rinses to column, allowing solvent to drain to packing level in between rinses.

7.7.2. As last rinse approaches top of adsorbent layer, add 70 mL of DCM/hexane (1:1) and allow elution to proceed at ca 5 mL/min.

7.7.3. Concentrate the eluate to less than 2 mL with rotary evaporator and quantitatively transfer to a pear-shaped flask and concentrate to less than 0.5 mL with rotary evaporator. Quantitatively transfer in a 1 mL reacti-vial, rinsing the flask with iso-octane.

7.7.4. Further concentrate the eluate from the milk, low-lipid and small biopsies samples to less than 100 µL under a gentle nitrogen flow and to less than 200 µL for the large adipose tissues.

7.7.5. Quantitatively transfer the sample (with a Pasteur pipet) to a 500 µL Reacti-vial previously marked at the 270 µL level for the large adipose tissue samples and to the 170 µL for the others. Rinse flask with a few drops of iso-octane, adding the rinses to the vial.

7.7.6. Add exactly 20 µL $^{13}C_{12}$-PCB-138 normalization standard to the Reacti-vial using an Eppendorf pipet. Adjust the final volume to the 170 or 270 µL mark with iso-octane. ***Note:*** Exact volume is not critical, since normalization standard is used to quantify residue levels.

7.7.7. Cap and vortex until thoroughly mixed. The sample is ready to inject into the GC/MS (see Section 7.13.3).

7.8. GC Operating Conditions

7.8.1. *Column*

See Section 7.6.1 of MET-CHEM-OC-04.

7.8.2. *Injection information*

See Section 7.6.2 of MET-CHEM-OC-04.

7.8.3. *Oven temperature programme*

- 100°C, hold 3 min; 20°C /min to 180°C; 5°C /min to 300°C

7.8.4. *Carrier gas (He)*

See Section 7.6.4 of MET-CHEM-OC-04

7.9. MSD Operating Conditions

See Section 7.7.1 of MET-CHEM-OC-04

7.10. Data Analysis Parameters

- Calibration settings — Reference window: 10.00%
- Non-reference window — 5.00%
- Default multiplier — 1.00
- Default sample concentration — 1.00

7.11. Compound Information

Chromatographic windows and characteristic ions are given in **Table 1**. Retention time, target ion and qualifying ion are given in **Table 2**. The list is based on those compounds actually found in polar bear tissues using PBQ2 as a secondary standard.

Table 1. Chromatographic windows and characteristic ions

SIM Group	Start time (min)	Dwell time (ms)	Ions
Group 1	7.40	60	216, 224 [a]
Group 2	9.40	60	250, 258 [a]
Group 3	10.20	60	183, 219, 250, 284, 292 [a]
Group 4	13.00	60	238, 268 [a], 292, 304 [a]
Group 5	16.00	60	255, 291, 292, 308, 339, 353, 378, 387
Group 6	17.60	60	292, 326, 339, 373, 375
Group 7	18.40	60	246., 326, 373, 375, 409
Group 8	19.00	60	246, 326, 360, 380
Group 9	20.10	60	235, 237, 326, 338 [a], 360, 443,
Group 10	20.70	60	235, 237, 326, 360, 372 [a], 394,
Group 11	21.70	60	235, 237, 360, 394,
Group 12	23.50	60	360, 394, 406 [a]
Group 13	25.80	60	251, 311, 430, 442 [a], 464, 498

[a] ions selected to monitor the ^{13}C-labeled internal standards

TABLE 2 - Retention time and target ions for secondary quantitative standard (PBQ2) and internal standard

Compound [a]	RT [b] (min)	RT window	Tgt [c] (m/z)	QI [d]	Abundance ratio [e]
Quantitation standard (PBQ2)					
1,2,4,5-tetrachlorobenzene	7.71	7.31 - 8.11	216	214	77
pentachlorobenzene	9.75	9.35 - 10.15	250	252	65
hexachlorobenzene	12.15	11.75 - 12.55	284	286	81
alpha-hexachlorocyclohexane	11.9	11.5 - 12.3	219	183	80
beta-hexachlorocyclohexane	12.55	12.15 - 12.85	219	183	119
octachlorostyrene	17.11	16.71 - 17.51	308	380	42
compound C	14.31	13.91 - 14.71	238	373	
U-3	10.47	10.07 - 10.87	250	252	85
photoheptachlor	16.62	16.22 - 17.02	339		
heptachlor epoxide	17.27	16.87 - 17.67	353	237	
oxychlordane	17.32	16.92 - 17.72	387	119	
U-4	17.48	17.08 - 17.88	255	291	34
C-5	17.87	17.62 - 18.12	373	375	96
C-3	17.89	17.49 - 18.29	339		
C-4	18.25	17.85 - 18.65	373	375	89
nonachlor III	18.57	18.27 - 18.87	409	407	88
t-nonachlor	18.75	18.45 - 19.05	409	407	88
U-2	20.39	19.99 - 20.79	443		
dieldrin	19.39	18.99 - 19.79	380	308	
p,p'-DDE	19.21	18.81 - 19.61	246	318	95
p,p'-DDD	20.65	20.25 - 21.05	235	237	42
p,p'-DDT	21.99	21.59 - 22.39	235	237	42
PCB-47/48	15.64	15.24 - 16.04	292	290	77
PCB-74	17.22	16.82 - 17.62	292	290	77
PCB-56/60	18.08	17.68 - 18.48	292	290	77
PCB-99	18.43	18.23 - 18.83	326	328	65
PCB-85	19.32	18.92 - 19.72	326	328	65
PCB-154	19.53	19.13 - 19.93	360	362	81
PCB-149	20.35	19.95 - 20.75	360	362	81
PCB-118	20.40	20.00 - 20.8	326	328	65
PCB-146	20.97	20.57 - 21.37	360	362	81
PCB-153	21.19	20.79 - 21.59	360	362	81
PCB-105	21.34	20.94 - 21.49	326	328	65
PCB-137	21.89	21.49 - 22.29	360	362	81
PCB-138/163	22.16	21.76 - 22.56	360	362	81
PCB-187	22.76	22.36 - 23.16	394	396	97
PCB-183	22.94	22.54 - 23.34	394	396	97
PCB-156	23.97	23.57 - 24.37	360	362	81
PCB-157	24.17	23.77 - 24.57	360	362	81
PCB-180	24.55	24.15 - 24.95	394	396	97
PCB-170/190	25.6	25.20 - 26.00	394	396	97
PCB-195	27.16	26.66 - 27.76	430	428	88
PCB-194	27.82	27.42 - 28.22	430	428	88
PCB-206	29.14	28.74 - 29.54	464		
PCB-209	30.20	29.60 - 30.80	498		

cont'd

TABLE 2 - continued

	Compound [a]	RT [b] (min)	RT window	Tgt [c] (m/z)	QI [d]	Abundance ratio [e]
Internal standard						
	$^{13}C_6$-1,2,4,5-tetrachlorobenzene	7.71	7.31 - 8.11	224		
	$^{13}C_6$-1,2,4,5-pentachlorobenzene	9.75	9.35 - 10.15	258		
	$^{13}C_6$-1,2,4,5-hexachlorobenzene	12.15	11.75 - 12.55	292		
	$^{13}C_{12}$-PCB-28	14.35	13.95 - 14.75	268		
	$^{13}C_{12}$-PCB-52	15.39	14.99 - 15.79	304		
	$^{13}C_{12}$-PCB-118	20.4	20.00 - 20.80	338		
	$^{13}C_{12}$-PCB-153	21.18	20.78 - 21.58	372		
	$^{13}C_{12}$-PCB-180	24.54	24.14 - 24.94	406		
	$^{13}C_{12}$-PCB-194	27.82	27.42 - 28.22	442		

[a] Abbreviations:

compound C - unknown structure

U2, U3, U4 - unknown chlorinated compounds

C3,C4, C5 - unknown chlordane related compounds

DDE - dichloro diphenyl dichloro ethylene

DDD - dichloro diphenyl dichloro ethane

DDT - dichloro diphenyl trichloro ethane

PCB - polychlorinated biphenyl

[b] Retention time. Temperature program: 100°C, hold 3 min; 20°C/min to 180°C; 5°C/min to 300°C

[c] Target ion - Mass (m/z)

[d] Qualifying ion

[e] Abundance ratio - applicable only to ions within molecular ion clusters

7.12. MS Tuning

See Section 7.10 of MET-CHEM-OC-04.

7.13. Analysis of Standards and Samples

7.13.1. *Quantitative standard for milk samples, adipose tissues from biopsy and low-lipid samples* - In a 200 µL insert of an autosampler vial, combine 140 µL of the quantitative standard PBQ with 20 µL of the ^{13}C-labeled chlorobenzenes/PCBs and 10 µL of the normalization standard $^{13}C_{12}$-PCB-138, for a final volume of 170 µL. Cap the vial and vortex to ensure mixing. ***Note:*** Prepared just prior to injection.

7.13.2. *Quantitative standard for larger adipose tissue sample* - In a 300 µL insert of an autosampler vial, combine 240 µL of the quantitative standard PBQ with 20 µL of the ^{13}C-labeled chlorobenzenes/PCBs and 10 µL of the normalization standard $^{13}C_{12}$-PCB-138, for a final volume of 270 µL. Cap the vial and vortex to ensure mixing. ***Note:*** Prepared just prior to injection.

7.13.3. *Samples* - Transfer the spiked sample from Section 7.7.7 into a 200 (or 300) µL insert of an autosampler vial and cap. Store in the dark at room temperature until ready for analysis. If analysis will not be performed on the same day, store the vial at 0-4°C.

7.13.4. Establish the operating conditions given in Sections 7.8-7.9.

7.13.5. Prepare sequence injection table as described in SOP-CHEM-PROC-10. A typical sequence would consist of the following solutions:

- PBQ quantitation standard (7.13.1 or 7.13.2)
- Method blank
- Samples (a series of 5)
- PBQ quantitation standard (7.13.1 or 7.13.2)

8. EXPRESSION OF RESULTS

8.1. Calculation of Analyte Concentration

$$C_S = [(A_S / A_{PBQ}) \times C_{PBQ}] \times [(V_{PBQI} / V_{PBQF})] \times [(A_{NS\ in\ PBQ} / A_{NS\ in\ S})] \times [(V_{SF} / W_S)] / 10^{-6}$$

where: C_S = analyte concentration in the sample in µg/g (wet weight)

A_S = area counts of analyte in the sample

A_{PBQ} = area counts of analyte in the PBQ injected standard

C_{PBQ} = analyte concentration in the PBQ standard solution in pg/µL

V_{PBQI} = initial volume of the PBQ standard solution used to prepare the quantitation standard mixture, in µL

V_{PBQF} = final volume of the PBQ quantitation standard mixture, in µL

V_{SF} = final volume of the sample, in µL

$A_{NS\ in\ PBQ}$ = area counts of $^{13}C_{12}$-PCB-138 normalization standard in the PBQ standard solution

$A_{NS\ in\ S}$ = area counts of $^{13}C_{12}$-PCB-138 normalization standard in the sample

W_S = sample weight in g (wet weight)

10^{-6} to convert in µg/g

Note: A Microsoft Excel spreadsheet is used to automatically calculate these values following sample acquisition on the GC/MSD.

8.2. Calculation of Recovery for Labeled Internal Standards

The percent recovery (%R) of labeled compounds is calculated as follows:

$$\%R = (A_{IS\ in\ S} / A_{IS\ in\ PBQ}) \times (A_{NS\ in\ S} / A_{NS\ in\ PBQ})$$

where: $A_{IS\ in\ S}$ = area counts of the labeled internal standard in the sample

$A_{IS\ in\ PBQ}$ = area counts of the labeled internal standard in the quantitation standard mixture

$A_{NS\ in\ S}$ = area counts of $^{13}C_{12}$-PCB-138 normalization standard in the sample

$A_{NS\ in\ PBQ}$ = area counts of $^{13}C_{12}$-PCB-138 normalization standard in the quantitation standard mixture

Note: Recoveries are automatically calculated by the instrument.

9. REPRESENTATIVE DOCUMENTS

TABLE 3 - Results table / Polar bear liver

File Name PBIS3W38.D
Path C:\HP3D\ESTN\DATA\COURTNEY\PBIS3W\
Date Acquired 15 Dec 99 5:15 am
Operator Courtney Sandau
Method File PBQ_NOV
Sample Nam PB X11782 (wt=5.23)
More Info
# of Compou 56
Last Cal. Upd Wed Dec 15 14:02:19 1999

Lipid % 1
Sample Weight (g) 5.23
New N.F. (138) 1.050

Cal. Std. File: 3Q quantitation
PBQ vol. uL 180
I.S. (2.5 ng/uL) uL 10
E.S. (5 ng/uL) uL 10
IS #2 DDE (2ng/uL) 10
Isooctane dilution uL 0
SAM F.V. uL 210

#	Compound	STD R.T (min.)	STD (conc.) (pg/uL)	STD (area)	SAM R.T. (min.)	SAM (area)	conc. (pg/uL)	Total Amount (pg)	Conc. w.w. (pg/g)	Rec.Cor (pg/g)
	Performance Standard									
56	C13 - PCB138 (ES)	19.95	222.38	205747	19.94	195881				
	Individual CHC Concentrations									
1	TeClBz	8.69	23	35892	8.73	49653	29	6018	1150.25	1813.19
2	PnClBz	8.48	40	49881	8.53	10435	6	1588	303.72	484.56
3	HCB	10.58	936	938047	10.60	10567	9	1994	381.17	552.00
4	a-HCH	10.46	114	103218	10.39	19780	20	4130	789.74	1143.70
5	g-HCH	11.06	29	24732	10.98	20852	22	4823	883.89	1280.03
6	OCS	15.13	14	6814	15.12	2468	5	988	188.85	273.49
7	Compound C	12.23	32	2514	12.24	5436	62	13089	2502.59	3624.23
8	Photoheptachlor	14.83	49	19211	14.83	9804	22	4875	893.83	1294.44
9	Heptachlor Epoxide	15.26	788	89791	15.38	8807	18	3367	643.70	932.29
10	Oxychlordane	15.30	728	82790	15.29	45615	361	75838	14500.27	20999.13
11	U-4	15.46	88	14012	15.45	16894	74	15501	2963.87	4292.24
12	C-5	16.17	13	10847	16.18	9397	10	2110	403.42	584.23
13	C-3	15.84	18	20703	15.84	21663	17	3564	681.51	986.95
14	C-4	16.46	15	8786	16.45	3321	5	1071	204.73	296.48
15	Nonachlor III	16.46	205	132459	16.46	43037	80	12593	2407.83	3486.99
16	t - Nonachlor	16.84	312	184224	16.84	23421	40	8413	1608.56	2329.49
17	U-2	18.21	48	13896	18.18	5437	17	3603	688.91	997.67
18	Dieldrin	17.29	564	39973	17.22	1179	20	4237	810.11	1173.18
19	p,p'-DDE	17.13	694	860754	17.12	32995	24	5090	961.70	1392.73
20	p,p'-DDD	18.50	29	33320	18.50	1323	1	218	41.63	60.28
21	p,p'-DDT	19.78	47	67052	19.78	2895	2	384	73.35	106.22
22	PCB 52	13.56	n/a	18858	13.54	4073	12	2491	476.28	542.77
23	PCB 47/48	13.77	61	79578	13.77	9881	7	1432	273.82	312.04
24	PCB 74	15.25	37	53400	15.22	4577	3	600	114.64	130.65
25	PCB 56/60	16.02	11	2819	15.99	576	2	426	81.54	92.92
26	PCB 101	16.21	n/a	39026	16.20	8002	10	2132	407.68	464.59
27	PCB 99	16.39	710	748099	16.37	148819	128	26775	5119.56	5834.20
28	PCB 85	17.23	55	80735	17.21	8249	7	1412	270.04	307.74
30	PCB 149	18.19	13	9552	18.20	3423	4	881	168.41	191.91
31	PCB 118	18.26	130	148375	18.25	11453	9	1897	362.76	413.39
32	PCB 146	18.61	59	42472	18.60	8560	11	2248	429.87	489.86
33	PCB 153	19.02	3222	2281430	19.01	518232	659	138375	26457.66	30151.16
34	PCB 105	19.15	36	36652	19.13	4133	4	788	148.75	167.24
35	PCB 137	19.68	43	28211	19.67	8375	12	2548	486.88	566.02
36	PCB 138/163	19.95	817	432934	19.94	85954	110	23180	4426.35	5046.51
37	PCB 187	20.54	40	19295	20.52	3454	6	1356	259.26	295.44
38	PCB 183	20.70	36	19094	20.69	7375	13	2629	502.73	572.91
39	PCB 156	21.70	34	27052	21.71	9514	11	2261	432.28	492.62
40	PCB 157	21.90	34	25229	21.90	7947	10	2025	387.18	441.21
41	PCB 180	22.28	1225	632830	22.32	160855	280	58797	11242.30	12811.63
42	PCB 170/190	23.28	622	314908	23.44	60614	108	22636	4328.05	4932.21
43	PCB 195	23.78	10	4872	23.90	2211	4	858	164.06	186.96
44	PCB 194	25.48	212	80895	25.70	10802	25	5352	1023.37	1166.22
45	PCB 206	26.77	20	13452	26.95	3368	5	947	181.02	206.29
46	PCB 209	27.81	23.5	3587	27.89	769	5	953	182.12	207.55

Percent Recoveries, Interna' Standards

#	Compound	INT FILE	STD R.T (min.)	STD (conc.) (ng/uL)	STD (area)	SAM R.T. (min.)	SAM (area)	Recovery %		Total	rec corr. Total	
										1835.14	2788.76	sum ClBzs
										8753	1674	sum HCHs
										1078.68	1556.23	sum DD?
									avgClBz	27044.36	39155.35	sum OCs
47	13C-TeClBz		8.69	119.05	107114	8.73	64892	63.4	99.2			
48	13C-PnClBz		8.48	119.05	109564	13.77	78331	75.1		57838.82	88624.04	sum PCBs
49	13C-HCB		10.58	119.05	96841	15.22	63664	69.1				
50	13C-PCB 28		12.60	93.81	111498	12.59	100293	94.5				
51	13C-PCB 52		13.53	95.23	122901	13.54	103470	88.4	mean PCB			
52	13C-PCB 118		18.28	100.24	118158	18.24	102368	91.0	% recovery			
53	13C-PCB 153		19.02	102.62	79049	19.00	65027	86.4	87.8			
54	13C-PCB 180		22.28	96.1	31567	22.31	29139	93.8				
55	13C-PCB 194		25.48	98.57	39042	25.68	26869	72.8				
57	C13 - B-HCH		11.06	95.2	24283	10.98	17745	76.8				
58	C13 - DDE		17.16	95.2	135830	17.11	119212	92.3				

Init. Conc. = (Std. Conc.*(N.F.)*(pk. area in Sam./pk. area in Std.))/1000 = ng/uN.F. = peak area(CB154(std.)/CB154(sam.))
Total Amt. = Init. Conc.*(5m$4/5m$7)*(F.V. ul of sample) = ng
Conc. w.w. = (Total Amt /Sam. wt.) = ng/g
Conc. l.w. = Conc. w.w./(% lipid/100) = ng/g

10. QUALITY CONTROL

10.1. MSD Tuning

See Section 10.1 of MET-CHEM-OC-04.

10.2. Calibration Verification

A four-point initial calibration standard curve is generated every six months for the major compounds (e.g., oxychlordane, PCB-153, etc.) found in the polar bear quality control material (PBQ) to cover the range of interest. This established calibration curve is verified daily, by analyzing a calibration verification standard (quantitation standard) having a mid-point concentration. The calculated concentration of each compound must be within 20% of its actual known value. The final concentration of any reportable compounds must be within the demonstrated linearity of the detector. If necessary, samples are diluted with iso-octane to meet the calibration range.

10.3. Detection Limit

Minimum detectable concentration (MDC) is the concentration of analyte which produces a signal 3 times the average noise level. Detection limit, using MSD, is highly variable with compound since background noise and response factors are different for each ion monitored. The background noise is affected by several factors, such as tissue size and tissue type, instrument (column bleed and cleanliness of the source on the day of the analysis). Typically a detection limit of 0.001 ppm (or less) is achievable. MDC for individual compounds are calculated periodically using the noise calculation function of the ChemStation software.

10.4. Ongoing Precision and Recovery

An aliquot of a PBQ Reference Material is analyzed along with each batch of samples. The concentration of the major compounds is determined and the results are compared to the previously established acceptance limits (i.e., ± 2 SD of the long-term mean plotted in a Shewart chart - ref. SOP-CHEM-DOC-02).

To determine the degree of analyte loss during sample cleanup, each sample (including the PBQ reference material) is spiked with ^{13}C-labeled chlorobenzenes/PCBs internal standard mixture. It is assumed that during

sample cleanup, native compounds behave the same way as the labeled compounds. Analysis is accepted when the % internal standard recoveries for most PCBs and OCs are between 80% and 110%, and for the highly volatile compounds (eluting in the 1st, 2nd and 3rd chromatographic windows) are over 60%. The levels of contaminants reported in the analytical test report are not corrected for internal standard recoveries, but the recovery data are included.

10.5. Accuracy

To improve the accuracy of the method, the compound levels can be corrected based on the recovery of the ^{13}C-labeled chlorobenzenes/PCBs internal standard mixture (4.6.1). The majority of the chlorinated pesticides and PCB congeners levels can be corrected based on the average recovery of the five higher chlorinated ^{13}C-labeled PCB standards (i.e., PCB-52, PCB-118, PCB-153, PCB-180 and PCB-194). The more volatile compounds can be recovery-corrected using the recovery of their corresponding ^{13}C-labeled standards. For volatile compounds without ^{13}C-surrogates, retention time interpolation of recoveries with the ^{13}C-standards can be used.

The accuracy of the quantitation standards is verified annually with a second source standard (containing most of the congeners of interest) as described in SOP-CHEM-PROC-13.

10.6. Method Blank

See Section 10.6 of MET-CHEM-OC-04.

10.7. Target Compound Identification

See Section 10.7 of MET-CHEM-OC-04.

10.8. Standard Operating Procedures

See Section 10.8 of MET-CHEM-OC-04.

10.9. Data Validation

See Section 10.9 of MET-CHEM-OC-04.

10.10. External Quality Control

As part of its quality-assurance effort, the Laboratory organized an inter-laboratory study involving the determination of 33 compounds in a polar bear fat sample. Results obtained in our laboratory were in good agreement with the results in the other five participant laboratories (average of 35% variation for the 33 compounds examined) [2.9].

11. Critical Control Points

See Section 11 of MET-CHEM-OC-04 and:

The flow of nitrogen (used to concentrate the extracts) has to be adjusted so that the surface of the solvent is just visibly disturbed and the sample must never be brought to dryness as it may cause analyte loss.

Analysts must be aware that electronic integration of data has its pitfalls. Examination of chromatograms and results by a competent analyst is essential.

When doing timed activities ensure each sample is treated equally (e.g., when doing moisture or lipid determinations).

APPENDIX A

Numbering of PCB Congeners

Numbering of PCB congeners - Adapted from Ballschmiter, K. *et al.* (1980) Anal. Chem., 302, 20-31.

Monochlorobiphenyls

1	2
2	3
3	4

Dichlorobiphenyls

4	2,2'
5	2,3
6	2,3'
7	2,4
8	2,4'
9	2,5
10	2,6
11	3,3'
12	3,4
13	3,4'
14	3,5
15	4,4'

Trichlorobiphenyls

16	2,2',3
17	2,2',4
18	2,2',5
19	2,2',6
20	2,3,3'
21	2,3,4
22	2,3,4'
23	2,3,5
24	2,3,6
25	2,3',4
26	2,3',5
27	2,3',6
28	2,4,4'
29	2,4,5
30	2,4,6
31	2,4',5
32	2,4',6
33	2',3,4
34	2',3,5
35	3,3',4
36	3,3',5
37	3,4,4'
38	3,4,5
39	3,4',5

Tetrachlorobiphenyls

40	2,2',3,3'
41	2,2',3,4
42	2,2',3,4'
43	2,2',3,5
44	2,2',3,5'
45	2,2',3,6
46	2,2',3,6'
47	2,2',4,4'
48	2,2',4,5
49	2,2',4,5'
50	2,2',4,6
51	2,2',4,6'
52	2,2',5,5'
53	2,2',5,6'
54	2,2',6,6'
55	2,3,3',4
56	2,3,3',4'
57	2,3,3',5
58	2,3,3',5'
59	2,3,3',6
60	2,3,4,4'
61	2,3,4,5
62	2,3,4,6
63	2,3,4',5
64	2,3,4',6
65	2,3,5,6
66	2,3',4,4'
67	2,3',4,5
68	2,3',4,5'
69	2,3',4,6
70	2,3',4',5
71	2,3',4',6
72	2,3',5,5'
73	2,3',5',6
74	2,4,4',5
75	2,4,4',6
76	2',3,4,5
77	3,3',4,4'
78	3,3',4,5
79	3,3',4,5'
80	3,3',5,5'
81	3,4,4',5

Pentachlorobiphenyls

82	2,2',3,3',4
83	2,2',3,3',5
84	2,2',3,3',6
85	2,2',3,4,4'
86	2,2',3,4,5
87	2,2',3,4,5'
88	2,2',3,4,6
89	2,2',3,4,6'
90	2,2',3,4',5
91	2,2',3,4',6
92	2,2',3,5,5'
93	2,2',3,5,6
94	2,2',3,5,6'
95	2,2',3,5',6
96	2,2',3,6,6'
97	2,2',3',4,5
98	2,2',3',4,6
99	2,2',4,4',5
100	2,2',4,4',6
101	2,2',4,5,5'
102	2,2',4,5,6'
103	2,2',4,5',6
104	2,2',4,6,6'
105	2,3,3',4,4'
106	2,3,3',4,5
107	2,3,3',4',5
108	2,3,3',4,5'
109	2,3,3',4,6
110	2,3,3',4',6
111	2,3,3',5,5'
112	2,3,3',5,6
113	2,3,3',5',6
114	2,3,4,4',5
115	2,3,4,4',6
116	2,3,4,5,6
117	2,3,4',5,6
118	2,3',4,4',5
119	2,3',4,4',6
120	2,3',4,5,5'
121	2,3',4,5',6
122	2',3,3',4,5
123	2',3,4,4',5
124	2',3,4,5,5'
125	2',3,4,5,6'
126	3,3',4,4',5
127	3,3',4,5,5'

Hexachlorobiphenyls

128	2,2',3,3',4,4'
129	2,2',3,3',4,5
130	2,2',3,3',4,5'
131	2,2',3,3',4,6
132	2,2',3,3',4,6'
133	2,2',3,3',5,5'
134	2,2',3,3',5,6
135	2,2',3,3',5,6'
136	2,2',3,3',6,6'
137	2,2',3,4,4',5
138	2,2',3,4,4',5'
139	2,2',3,4,4',6
140	2,2',3,4,4',6'
141	2,2',3,4,5,5'
142	2,2',3,4,5,6
143	2,2',3,4,5,6'
144	2,2',3,4,5',6
145	2,2',3,4,6,6'
146	2,2',3,4',5,5'
147	2,2',3,4',5,6
148	2,2',3,4',5,6'
149	2,2',3,4',5',6
150	2,2',3,4',6,6'
151	2,2',3,5,5',6
152	2,2',3,5,6,6'
153	2,2',4,4',5,5'
154	2,2',4,4',5,6'
155	2,2',4,4',6,6'
156	2,3,3',4,4',5
157	2,3,3',4,4',5'
158	2,3,3',4,4',6
159	2,3,3',4,5,5'
160	2,3,3',4,5,6
161	2,3,3',4,5',6
162	2,3,3',4',5,5'
163	2,3,3',4',5,6
164	2,3,3',4',5',6
165	2,3,3',5,5',6
166	2,3,4,4',5,6
167	2,3',4,4',5,5'
168	2,3',4,4',5',6
169	3,3',4,4',5,5'

Heptachlorobiphenyls

170	2,2',3,3',4,4',5
171	2,2',3,3',4,4',6
172	2,2',3,3',4,5,5'
173	2,2',3,3',4,5,6
174	2,2',3,3',4,5,6'
175	2,2',3,3',4,5',6
176	2,2',3,3',4,6,6'
177	2,2',3,3',4',5,6
178	2,2',3,3',5,5',6
179	2,2',3,3',5,6,6'
180	2,2',3,4,4',5,5'
181	2,2',3,4,4',5,6
182	2,2',3,4,4',5,6'
183	2,2',3,4,4',5',6
184	2,2',3,4,4',6,6'
185	2,2',3,4,5,5',6
186	2,2',3,4,5,6,6'
187	2,2',3,4',5,5',6
188	2,2',3,4',5,6,6'
189	2,3,3',4,4',5,5'
190	2,3,3',4,4',5,6
191	2,3,3',4,4',5',6
192	2,3,3',4,5,5',6
193	2,3,3',4',5,5',6

Octachlorobiphenyls

194	2,2',3,3',4,4',5,5'
195	2,2',3,3',4,4',5,6
196	2,2',3,3',4,4',5,6'
197	2,2',3,3',4,4',6,6'
198	2,2',3,3',4,5,5',6
199	2,2',3,3',4,5,6,6'
200	2,2',3,3',4,5',6,6'
201	2,2',3,3',4,5,5',6'
202	2,2',3,3',5,5',6,6'
203	2,2',3,4,4',5,5',6
204	2,2',3,4,4',5,6,6'
205	2,3,3',4,4',5,5',6

Nonachlorobiphenyls

206	2,2',3,3',4,4',5,5',6
207	2,2',3,3',4,4',5,6,6'
208	2,2',3,3',4,5,5',6,6'

Decachlorobiphenyl

209	2,2',3,3',4,4',5,5',6,6'